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(54) Title: FOOD PRODUCTS COMPRISING SOY PROTEIN

(57) Abstract: The present invention concerns food products containing soy protein in a form which result in maximum cholesterol-lowering when ingested and also concerns methods for manufacturing said food products containing soy protein. Furthermore, the present invention relates to food products containing soy protein suitable for preventing, treating and/or alleviating cardiovascular diseases such as hypercholesterolemia, hypertriglyceridemia, hyperlipidemia, arteriosclerosis, hypertension and related cardiovascular diseases, for preventing and/or treating type 2 diabetes and/or the metabolic syndrome, and for preventing, treating and/or alleviating pulmonary diseases. The present invention also pertains to the use of such food products containing soy protein in the prevention and/or treatment of a cardiovascular disease in a subject suffering from type 2 diabetes.

## FOOD PRODUCTS COMPRISING SOY PROTEIN

## FIELD OF THE INVENTION

The present invention concerns food products containing soy protein in a form which  
5 result in maximum cholesterol-lowering when ingested and also concerns methods for  
manufacturing said food products containing soy protein. Furthermore, the present  
invention relates to food products containing soy protein suitable for preventing,  
treating and/or alleviating cardiovascular diseases such as hypercholesterolemia,  
hypertriglyceridemia, hyperlipidemia, arteriosclerosis, hypertension and related  
10 cardiovascular diseases, for preventing and/or treating type 2 diabetes and/or the  
metabolic syndrome, and for preventing, treating and/or alleviating pulmonary  
diseases. The present invention also pertains to the use of such food products  
containing soy protein in the prevention and/or treatment of a cardiovascular disease  
in a subject suffering from type 2 diabetes.

15

A soy protein product according to the present invention is particularly useful in  
preventing and/or reducing the influx of triglycerides and/or cholesterol into the arterial  
wall and/or reducing the accumulation of cholesterol in the arterial wall of subjects at  
high risk for developing cardiovascular disease or subjects already suffering from a  
20 cardiovascular disease such as atherosclerosis or diabetic subjects. A soy protein  
product according to the present invention is also useful for lowering serum levels of  
total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or  
for increasing serum levels of HDL-cholesterol and/or for improving the serum  
HDL/LDL-ratio in subjects at risk for developing cardiovascular diseases and in  
25 subjects already suffering from an arteriosclerotic condition such as e.g.  
atherosclerosis or a related cardiovascular disease. A soy protein product according to  
the present invention is also useful in lowering serum levels of total cholesterol and/or  
LDL-cholesterol and/or triglycerides and/or glucose and/or increasing serum levels of  
HDL-cholesterol in diabetic subjects. A soy protein product according to the present  
30 invention is also useful in treating e.g. chronic obstructive pulmonary disease (COPD),  
inflammation of the airways, asthma, bronchoconstriction, bronchitis, and small  
airways disease.

In addition the present invention relates to the use of these food products containing  
35 soy protein as a functional food and/or in the manufacture of a functional food for  
treating a subject suffering from cardiovascular diseases, more particularly

hypercholesterolemia, hypertriglyceridemia, hyperlipidemia, arteriosclerosis, hypertension and/or related cardiovascular diseases. The present invention also relates to the use of these food products containing soy protein as a functional food and/or in the manufacture of a functional food for treating type 2 diabetes and/or the metabolic syndrome and/or a cardiovascular disease in a subject suffering from type 2 diabetes. Furthermore, the present invention also relates to the use of these food products containing soy protein as a functional food and/or in the manufacture of a functional food for treating a subject suffering from a pulmonary disease, more particularly chronic obstructive pulmonary disease (COPD), inflammation of the  
10   airways, asthma, bronchoconstriction, bronchitis, and/or small airways disease..

The present invention also concerns use of a soy protein product according to the present invention in the prevention and/or treatment of said diseases and disorders and for lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein in subjects. In addition, the present invention also  
15   provides methods for preventing, treating, prophylactically treating and/or alleviating by therapy said diseases and disorders.

#### BACKGROUND OF THE INVENTION

20   The field of functional foods has increased tremendously in recent years as the health awareness of the population in the industrialised parts of the world has gone up coincident with an increase in lifestyle related syndromes such as obesity, cardiovascular diseases and type II diabetes. Several studies show a significant correlation between the diet of a subject and the risk of the subject of contracting one  
25   or more of these diseases and syndromes. This has put the spotlight on the healthiness of everyday diet and as research into the field continues many items are added to the group of ingredients that should be present in a healthy diet.

Intake of soy protein, either alone or in combination with other soybean components,  
30   has been shown to have several beneficial effects on humans. These benefits include improvements in plasma lipid and lipoprotein concentrations (i.e. lower LDL cholesterol, lower triglycerides and possibly higher HDL cholesterol). The beneficial effects of soy protein on plasma lipoprotein concentrations have been recognised in 1999, by the U.S. Food and Drug Administration's approval of a health claim that "25 g  
35   of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of coronary heart disease".

Naturally, the soy protein could be consumed by eating soybeans, but this has little appeal, as soybeans are conceived by some to have an objectionable flavour and furthermore are not part of a traditional diet in the Western world. An alternative way of  
5 consuming soy protein is as part of a food product containing soy protein.

Soy protein is used as a food ingredient in the form of products such as soy flour, soy protein concentrate and isolated soy protein. In conventional processes for manufacturing the protein products, the beans are initially cracked, dehulled and  
10 flaked. In this process the material is also conditioned, e.g. by boiling them in water for 20-30 minutes to destroy most of the activity of the trypsin inhibitors. The resulting flakes are then extracted with an inert solvent, such as a hydrocarbon solvent, typically hexane, in one of several types of counter-current extraction systems to remove the  
15 soybean oil. The defatted flakes, which are the starting material for most commercial protein ingredients, have a protein content of approximately 50%. Moisture content has typically been reduced to 3 to 5% during this process. Any residual solvent may be removed by heat and vacuum, often by employing a deodorising vessel to enable live steam to contact the flakes to remove additional residual solvent. Conditions in the deodorising vessel can be adjusted to produce flakes of varying protein solubility  
20 depending on the end-use requirement.

Following milling of the defatted flakes, the soy protein isolates are typically produced by extracting the protein from defatted soybean flakes with water or a solution of water and ethanol at a pH of 6-11, at temperatures up to 80°C to 90°C. The extracted  
25 soybean flakes enter a first centrifuge to separate the soluble fraction from the insoluble fibrous residue. The resulting soluble fraction (containing extracted protein and oligosaccharides) is acidified to a pH of about 4,5 corresponding to the isoelectric point of the protein with a food-grade acid. A second centrifuge is used to separate the whey (which includes the soluble oligosaccharides) from the protein curd that typically  
30 involve cooling to below room temperature. The curd is then washed with water in a washing tank using any number of multiple washings. The washed curd is commonly neutralised to a pH of about 7 with water and an alkali such as sodium or calcium hydroxide in a neutralisation tank to make the product pH neutral.

35 Finally the protein curd is normally spray-dried in a dryer at about 150-170°C inlet air temperature and about 80-85°C outlet temperature, to yield the soy protein isolate or

soy proteinate having a moisture content of about four (4) to six (6)% and a soluble protein content greater than about 90%.

Such conventional processes are e.g. described in, "Simultaneous aqueous extraction of oil and protein from soybean: Mechanisms for process design, Rosentahl et al. Trans ChemE. Vol. 76, part C, Dec 1998 and in patents US5674548, US4307014 and US4296026, US4172828 and US3716372.

The resulting products from the conventional manufacturing processes have physical properties and flavour characteristics, which render them useful as food additives. In recent years a number of alternative manufacturing processes aimed at obtaining products with specific characteristics have been described. These are aimed at obtaining a product with certain specified characteristics such as good dispersion properties, low tendency to crystallise, a high content of phytoestrogens etc.

It has for some time been considered a fact that the content of isoflavones where directly involved in the cholesterol lowering effect of soy proteins. Consequently a lot of effort has been devoted to develop processes which reduce the loss of isoflavones. The focus on the isoflavone content has ultimately lead to isoflavones being added to soy protein products after processing.

US5994508 relates to a process for providing an isoflavone rich protein isolate, along with the isoflavone rich protein isolate produced thereby. A vegetable material containing protein and at least one isoflavone compound is extracted with an aqueous extractant having a neutral pH. The pH of the extract is adjusted to about the isoelectric point of the protein, in order to precipitate the protein. Following cooling to about 5°C to about 25°C, the protein is separated from the extract. The cool separation temperatures increase the concentration of isoflavones recovered in the protein, while the neutral pH inhibits loss of protein normally observed at cool or cold separation temperatures.

US6013771 relates to a process for providing an isoflavone rich protein isolate, along with the isoflavone rich protein isolate produced thereby. A vegetable material containing protein and at least one isoflavone compound is extracted with an aqueous extractant having a pH above the isoelectric point of the protein, and preferably an alkaline pH. The pH of the extract is adjusted to about the isoelectric point of the

protein in order to precipitate the protein. Following cooling to about 5°C to about 25°C, the protein is separated from the extract. Washing of the separated protein is avoided, or is conducted with minimum amounts of water. The cool separation temperatures and the low wash conditions significantly increase the concentrations of  
5 isoflavones recovered in the protein.

US6140469 relates to the production of an isoflavone enriched vegetable protein isolate in which the weight ratio of material to extractant is controlled and washing of the acid precipitated protein curd is avoided or minimised to provide an increased level  
10 of isoflavones in the protein isolate.

Similar processes are further described in e.g. US6140469, US6132795, US6083553, US6015785, US6015771, US5994508, US5994508, US5990291 and US5919921. Isolates or extracts having a high fixed level of phytoestrogens/isoflavons are  
15 described in e.g. US6140469, US6015785, US6013771, US5994508, US573389, US5637562, US5726034 and US5637561. In most of these methods the resulting soy protein is totally denatured and the globular structure completely unfolded.

In recent years the theory that isoflavones are responsible for the cholesterol lowering  
20 effect has been questioned and today evidence that protein components are directly involved has been obtained. For example have Sirtori et al., shown that soy protein or fragments without isoflavones can lower cholesterol levels. This is supported by the demonstration, in major clinical investigations on hypercholesterolemic patients, that marked plasma cholesterol reduction was obtained using isoflavone-poor soybean  
25 products. Studies in humans and in animal models have furthermore suggested that the mechanism of soy protein might be linked to the direct activation of LDL receptors in liver cells, or to a modulation of the synthesis and catabolism of LDL by specific dietary amino acids. Such an activation has been demonstrated *in vitro* with both isoflavone-poor soy concentrate, isolated protein subunits  $\alpha$  and  $\alpha'$  from the 7S soy  
30 globulin and synthetic peptides derived from the  $\alpha/\alpha'$  sequence in liver cells.

The manufacturing processes for producing soy proteins have thus far not been concerned with what effect the process might have on the proteins with regard to their cholesterol lowering effect. This is probably due to the fact that soy proteins are  
35 believed to be relatively heat-stable. Furthermore, the cholesterol-lowering effect attributed to the proteins themselves or part thereof has been considered as being

independent on the conditions by which food products containing soy protein were manufactured.

However, a number of disclosures concern methods aimed at obtaining proteins in a not completely denatured due to the effect of the degree of denaturation on diverse physical-chemical properties. Examples of these are:

US6005076 which relates to processes for the preparation and purification of proteinaceous materials from oil seeds and protein meals in a substantially non-denatured form. A process is provided in which a protein isolate is obtained from fat-contaminated oil seed material by carrying out an aqueous salt extraction to solubilise protein and fat and subsequently removing the fat by applying a chilling process. Protein is retrieved as micelles upon decreasing the ionic strength of the solution. The resulting protein is described as having improved functional properties.

US5674548 which relates to soybean milk having a high transparency and a soybean protein material having excellent oil retention and minimised mouthfeel change and also relates to soybean protein providing transparent solutions and a strong and transparent gel. The process involves an aqueous protein extraction step in which the temperature is kept below 40°C. The proteins obtained are characterised in that 85% of the protein is able to permeate a 0,22µ membrane and by optical density at 600 nm.

US4737014 relates to a process for isolation of proteins from soybeans with high yield and in a substantially undenatured form. The process involves an aqueous extraction step in which the pH is adjusted to from about 4.8 to about 5.4 prior to dilution. The protein obtained is substantially undenatured as opposed to proteins obtained by conventional isoelectric precipitation and the yield is better than described in the state of the art.

US5936069 provides a method for producing an improved soy protein concentrate having low raffinose, stachyose; and fiber content, as well as minimal lipoxxygenase activity and/or good flavour. The improved soy protein concentrate is high in naturally occurring vitamins, minerals and isoflavones, and may also be high in phytoestrogens. The resulting soy protein concentrate has a protein content of about 60% to about 80% of available protein. Further, the improved soy protein concentrate contains less than 0.3% fibre. Also, since it is not necessary to use any alcohol or acid to produce



the improved soy protein concentrate, the final product has not been denatured and is highly functional. The final product is high in water-soluble minerals, proteins and isoflavones, and may generally be higher in phytoestrogens.

- 5 In none of the above references is any other means of determining the degree of denaturation than differential scanning calorimetry (DSC) described. Furthermore, none of the above references describe the degree of denaturation of the obtained products, nor contemplates that this would have any effect on the potential cholesterol lowering effect or other health benefits obtainable by ingestion of the produced soy protein. It has furthermore not been questioned whether subsequent processing steps which would normally be considered to have an effect on protein structure, such as heat treatment, would affect e.g. the ability of the soy protein to lower cholesterol. This may be due to the fact that soy proteins have been considered to be heat-stable and due to early studies which showed that soy protein incorporated into baked muffins had a cholesterol lowering effect. One should however take into consideration that the conditions (i.e. for example temperature/humidity) in the internal part of a baked product may not cause complete denaturation of the proteins.

- It has now surprisingly been found that soy proteins can lose their ability to lower blood cholesterol and to promote other health benefits when ingested, if excessive heat or other denaturing conditions are applied in the manufacturing process of the soy protein product and/or in subsequent processes where the soy protein is for example incorporated into food products.

- 25 Thus, without wishing to be bound by specific theory it is currently believed that certain peptides, which are breakdown products of soy protein formed in the digestive tract, could thus be responsible for the reduction of plasma cholesterol. If the formation of these peptides is dependent on the three-dimensional structure of the protein, this structure will determine the cholesterol-lowering effect of ingesting the soy protein. Processes, which result in structural changes of the soy protein, may therefore compromise the health benefit of ingesting the protein.

- It is thus an aim of the present invention to provide food products containing soy protein in a form which retain the cholesterol-lowering effect, a process for the manufacture of said food products and use of said food products.

## SUMMARY OF THE INVENTION

The present invention provides food products containing soy protein which retain the ability to lower blood cholesterol levels upon ingestion, characterised by having more than a further specified minimum content of intact 7S ( $\alpha + \alpha' + \beta$ ) and/or 11S subunits

5 (A + B), a minimum 7S enthalpy peak or a minimum solubility.

The present invention further provides a method for manufacturing and /or conservation of food products containing soy protein which retain the ability to lower blood cholesterol upon ingestion, characterised by having more than a further

10 specified minimum content of intact 7S ( $\alpha + \alpha' + \beta$ ) and/or 11S subunits (A + B), a minimum 7S enthalpy peak or a minimum solubility as well as the products which is the result of said method. Said method is further characterised by process conditions which render the 7S and 11S subunits intact.

15 The present invention also provides the use of the above food products containing soy protein which retain the ability to lower blood cholesterol levels upon ingestion, characterised by minimum content of intact 7S ( $\alpha + \alpha' + \beta$ ) and/or 11S subunits (A + B), a minimum 7S enthalpy peak or a minimum solubility to lower blood cholesterol levels upon ingestion to obtain a health benefit.

20

The present invention further provides a method for manufacturing food products which retain the ability to lower blood cholesterol levels upon ingestion, comprising the use of a soy protein product with a further specified minimum content of intact 7S ( $\alpha + \alpha' + \beta$ ) and/or 11S subunits (A + B), a minimum 7S enthalpy peak or a minimum

25 solubility. The present invention furthermore provides the products which is the result of said method and the use of said products to lower blood cholesterol levels upon ingestion to obtain a health benefit.

Without wishing to be bound by theory it is believed that ingestion of soy proteins with  
30 minimum content of intact 7S ( $\alpha + \alpha' + \beta$ ) and/or 11S subunits (A + B), a minimum 7S enthalpy peak or a minimum solubility, facilitate the formation of breakdown products in the digestive tract, in the form of peptides which elicit an effect in the form of cholesterol lowering.

35 The globulins are the major part of the soy proteins. These can be divided in 7 S and 11 S globulins. The ratio between 7S and 11 S can vary with the cultivars from 0.5-2.0.

7 S is a glycoprotein. The main component is  $\beta$ -Conglycinin, which is a trimer, molecular mass of 150-200 kDa. Three main sub-units:  $\alpha'$  (72 kDa),  $\alpha$  (68 kDa) and  $\beta$  (52 kDa). Seen from a nutritional point of view, the  $\alpha'$  is the best due to the higher  
5 content of cysteine, methionine and tryptophan.

11S is a glycosylated protein, glycinin. It is a hexamer with a globular mass of 300-380 kDa composed of six identical subunits. Each sub-unit consists of an acidic polypeptide (A), 35 kDa and a basic polypeptide (B), 20 kDa. The two polypeptides are  
10 linked together by a disulfide bond.

Sirtori, Lovati & al have performed a number of experiments in a hepatoma cell line (HepG2), an in vitro model of human liver cells that are highly sensitive to factors regulating LDL receptor expression, cholesterol biosynthesis and breakdown. Their  
15 studies have identified that the peptide corresponding to positions 127-150 (or a part of it) in the  $\alpha'$  of the 7S globulin of the soy protein is the really active part responsible for the upgrading of the LDL receptors in the liver. Which part of this peptide is really the active peptide is not defined yet (Lovati, Sirtori & al, J Nutrition 2000, 130:2543-2549). No upgrading effects were found on the  $\alpha$  and the  $\beta$ -unit at all. This finding  
20 support the hypothesis that if one or more peptides from the  $\alpha'$  units can reach the liver after been exposed to the enzymes in the stomach and intestine, they should elicit a cholesterol-lowering effect. In addition also the peptide comprising amino acids 10-32 from 7S $\alpha'$  may be active in the upgrading of the LDL receptors in the liver.

25 Clinical trials on soy proteins have been performed over many years. But the reported reductions in LDL give broad variety in the results, in fact from around 6% for patients > 5.7 mmol/l cholesterol (Bakhit & al, 1994, American Institute of Nutrition) and % up to 22-23 % for patients high in cholesterol, > 7 mmol/l (Sirtori & al, Lancet, 1977 i:275-277). The Bakhit study used ISP, baked in muffins as the application from for the  
30 study. The Sirtori study used a textured soy protein.

A summary is found in the meta-analysis by James Anderson & al, (The New England Journal of Medicine, 1995, August 3, 276-282). This analysis was performed on 38 clinical studies. These studies (performed from 1975 to 1995) used either ISP or  
35 textured soy protein. The average reduction in LDL was in average 12.9 % (6.8- 24 % (for patients with high cholesterol levels)).

It is relevant also to mention the hypocholesterolemic effect is directly correlated to the patient's cholesterolemia. Minimal effect or little reductions occur at cholesterol levels of 6 mmol/l or less and with most benefits with cholesterol of greater than 7 mmol/l (

5 Sirtori, Current Atherosclerosis Reports 2001, 3: 47-53 and J. Anderson, meta-analysis 1995. A new meta-analysis made by J. Anderson, was reported in poster form at the International Symposium on the Role of Soy, November 4-7, 2001 in San Diego, California, USA. The new analysis conclude that regular intake of ISP's can decrease the LDL by 6.1 %. The figure is based on only ISP based clinical studies from the later

10 years. One other conclusion is that isoflavon poor soy protein is less effective than isoflavon-rich ISP in reducing serum LDL. This is probably very much related to the study shown in Arch of Internal Medicine, volume 159, September 27: 2070-2076, where Crouse III & al describe a clinical, double blind study on 156 patients. In addition to placebo the patients were given one of 5 daily diets, placebo 25 g of casein,

15 the rest 25 g of soy protein with 3, 27, 37 and 62 mg isoflavons (specified as aglycons). As glycosides these figures corresponds to (specified as mg isoflavons, glycoside forms per g soy protein) 0.2, 1.8, 2.5 and 4.2. The results were as average: 2.5 %, 2.5 %, 4.2 % and 6 % reduction in LDL to placebo. For the patients > 6.5 mmol/l in cholesterol level, the figures were: 3.8 %, 4.5 %, 8.3 % and 9.8 %. For

20 patients <6 mmol/l the reduction was not significant.

On the other hand have Sirtori and his group did an evaluation of the LDL-receptor stimulatory activity (hepatoma cell line (HepG2) of the major soy isoflavon, genistein, up to a concentration of 30 mg/l and failed to demonstrate any evident changes.

25 Isoflavons are known to be inhibitors of tyrosine kinase. This means that they regulate negatively the cellular LDL receptor activity, shown in different experimental systems (Grove & al, J Biochem 1991, 266: 18194-18199, ( Sirtori, Current Atherosclerosis Reports 2001)

30 Studies of the effect of ingesting isoflavons in man (separate from soy proteins) have failed to show any cholesterol lowering effect (Hodgson JM and al, 1998, J Nutr 128: 728-732; Hsu CS and al, 2001, J Reprod Med 46 : 221-226; Nestel and al, 1999, J Clin Endocrinol Metab 84: 895-898).

35 As clearly outlined by many research groups, heat treatments of soy proteins weaken the bonds that maintain their secondary and tertiary molecular structure. As thermal

denaturation occurs, the protein molecules expose to the solvent (water) the hydrophobic areas that are buried in their native confirmation. This structural change generates an aggregation process of the partially unfolded protein molecules as a consequence of the imbalance between the attractive and repulsive forces of particles.

- 5 At concentrations above a certain level, the aggregation process sends in the formation of a network. Therefore, any thermal history that changes the suspension from a stabilized colloidal state to the network formation give rise to the following sequences of events:

10 Denaturation ———→ Aggregation ———→ Gelation

(R.Schmidt, ACS Symposium Series 147, American Chem. Soc., Washington DC, 1981; A. Clark and Ross Murphy, Adv. Polym. Sci. 1987, 83: 57-192; J.M. Anguilera, Food Technology 1995, 40 (10): 83-89).

15

The unfolding of the proteins is accompanied by enthalpic changes and can easily be followed by differential scanning calorimetry (DSC), which is the most used technique. The protein aggregation can be followed by gel electrophoresis (SDS-PAGE). In addition the denaturation will adversely affect the water solubility of the proteins and  
20 this may be measured by standard techniques. By use of such techniques the effect of heat treatment on structure, through denaturation, aggregation and effect on the properties of the ISP, are studied (Petroccelli and Añón, J. Agric. Food Chem. 1991, 39: 1386-1391; 1991, 39: 1029-1032; 1994, 42: 2161-2169; 1995, 43: 3035-3041).

25

The denaturation temperature for both 7S and 11S is influenced by thermal energy, pH, water concentration and to some degree also ionic strength. The denaturation temperatures increase a lot with reduced water content in the suspension of the soy proteins. High and low pH will also reduce the denaturation temperature. The 7S protein is the most vulnerable and will normally start denaturation at around 70 °C for  
30 10 % dispersion in water; but approx. 130 °C at a moisture content of around 30 %. At 80 °C it will take only a few seconds before all 7S will be completely denatured and the structure unfolded (J. Renkema & al, J. of Biotechnology 2000, 79:223-230; S.Petrucelli and M. Añón, J. Agric. Food Chem., 1996, 44: 3005-3009; A. Morales and J.L. Kokini, J. Rheol. 1999, 43: 315-325).

35

All results from the clinical tests compared to the technical/physical data and knowledge, indicate clearly that the more thermal energy that is used in the production process, the more denatured the proteins will be (unfolding of structure, aggregation of the strands to bigger clusters of insoluble particles), the less probable it is that the

5 molecules will still have the "active" part of the molecule.

A test on commercial available ISPs before 1990, showed a broad variety of qualities regarding the denaturation status (Arrese, Wagner and Añón, J. Agric. Food Chem. 1991, 39: 1029-1032). A similar test on commercial available ISPs to day show that all

10 of them are completely denatured on the 7S protein part. Further heating as in for example preparation of muffins will worsen the possibility to have any  $\alpha'$  of the 7S intact.

It is quite clear that ISPs are generally much more denatured than for example normal

15 qualities of soy flour as these, before drying, is normally the starting point for manufacturing of the ISP. The normal raw material for texturing of soy protein, is soy flour. The less denatured your starting point is before further addition of thermal and mechanical energy, the less you will change the protein structure and the less 7S $\alpha'$  will be ruined.

20 In order to keep the ISP (or what ever type of soy product) at a high nutritional quality it is important to keep the Trypsin inhibitors (Bowman-Birk inhibitor and Kunitz Trypsin inhibitor) at a level of 5-20 % of the natural occurring in the soy bean (below 50 UTI/mg). There might be a compromise in reaching this level and at the same time denature the soy proteins as little as possible. It will definitely be important to use

25 lowest possible moisture in the heating process step of the manufacturing of the new and improved ISP. It is also important to avoid high pH at any steps in the production (not higher than pH 9.5).

According to a new surprising correlation we have found between the denaturing

30 status of the soy protein and the cholesterol lowering effect, it is questionable what effect - if any - the isoflavons could have. We believe that the correlation between effect and the level of isoflavons is mostly a consequence of the process conditions which are applied in the manufacture of the ISP. Process conditions which keep the soy protein in a relatively undenatured form also favour a high level of isoflavons. An

35 example is alcohol extraction which will increase unfolding and also dissolve not only the fat soluble ingredients, but also some of the most hydrophobic amino acids. It is

well known that in the folded protein structure, which is a disc in shape, the hydrophobic part is towards the inside of the disc, where the isoflavons are linked to this hydrophobic part of the amino acids by hydrogen bonds. Even in denatured 7S part of the soy protein, the hydrophobic part is still protected inside the protein molecules, still linked to the isoflavons. Used in this form and status, it is possible that the enzymes in the gastric and intestinal juices will not break up the polypeptides which are linked to the some part of the 7S part of the proteins.

Early preparations of soy protein, which were not subjected to the same processing conditions as are used today, had a much more pronounced soy flavour and odour. The product quality of the ISP's, which were developed in last 5-7 years, is good, almost neutral in flavour and odour and tailor made for specific applications (for liquid products, bars and powders). The producers deliberately change the structure to get the specific properties they want depending on the application. Some of the qualities are also enzymatic treated as part of the process to obtain the preferred properties.

The present invention for the first time offers a solution as to how high amounts of soy protein which has not been processed in a way which denature the soy proteins, can be incorporated in food products without resulting in objectionable flavour or odour.

A substantial knowledge has been obtained in industry (to a large extent as secret know how) and in scientific research programs in the universities. Studies have in particular been aimed at the effect of the different process parameters (heat, pH, temperature/time) on both structure and on how to achieve specific properties (Food Proteins and their Applications, F. Damodaran and A. Paraf, 1997, chapter 9: Structure-Function Relationships of Soy Proteins; Shigeru Utsumi, Yasuki Matsumura and Tomochiko Mori: 259-291, Marcel Dekker Inc, New York, ISBN: 0-8247-9820-1). (Hermansson & al, J. Texture Stud. 1978, 9: 33-58; Hermansson & al, J. Am. Oil Chem. Soc., 1986, 63: 658-666).

The term "processed soy protein product" as used throughout the present specification and the appended claims shall be taken to mean any product containing soy protein obtained by processing of soy beans.

The term "intact subunit" as used throughout the present specification and the appended claims shall be taken to mean a protein subunit which has the molecular

weight of the full-length protein subunit as well as a protein subunit which is associated with other subunits by e.g. disulfide bridges or non-covalent bonding. The content of such "intact subunits" can for example be performed by analysis by SDS-PAGE under reducing conditions.

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The terms "7S subunits" and "11S subunits" as used throughout the present specification and the appended claims shall be taken to mean the subunits which constitute the globulins in legume plants. These are in soy beans also referred to as  $\beta$ -conglycinin which is a trimer of  $\alpha$ ,  $\alpha'$  and  $\beta$  and glycinin which is a hexamer

10 comprising A and B subunits.

The term "ability to lower blood cholesterol" as used throughout the present specification and the appended claims shall be taken to mean being effective in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or in increasing the serum HDL/LDL-cholesterol ratio and/or increasing serum levels of high-density lipoproteins (HDL) and/or in generating a decrease in serum levels of low-density lipoproteins (LDL). It is desirable to achieve an elevated serum HDL/LDL-cholesterol ratio since this may result in an increased reverse cholesterol transport and a subsequent excretion.

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The term "health benefit" as used throughout the present specification and the appended claims shall be taken to mean any beneficial effect in the prevention or treatment of disease.

25 **DETAILED DESCRIPTION OF THE INVENTION**

In one aspect of the present invention provides food products containing soy protein which retain the ability to lower blood cholesterol levels upon ingestion, characterised by having more than a further specified minimum content of intact 7S ( $\alpha + \alpha' + \beta$ ) and/or 11S subunits (A + B), a minimum 7S enthalpy peak or a minimum solubility.

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In another aspect of the present invention further provides a method for manufacturing and /or conservation of food products containing soy protein which retain the ability to lower blood cholesterol upon ingestion, characterised by having more than a further specified minimum content of intact 7S ( $\alpha + \alpha' + \beta$ ) and/or 11S subunits (A + B), a minimum 7S enthalpy peak or a minimum solubility as well as the products which is

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the result of said method. Said method is further characterised by process conditions which render the 7S and 11S subunits intact.

5 In another aspect of the present invention also provides the use of the above food products containing soy protein which retain the ability to lower blood cholesterol levels upon ingestion, characterised by minimum content of intact 7S ( $\alpha + \alpha' + \beta$ ) and/or 11S subunits (A + B), a minimum 7S enthalpy peak or a minimum solubility to lower blood cholesterol levels upon ingestion to obtain a health benefit.

10 In another aspect of the present invention further provides a method for manufacturing food products which retain the ability to lower blood cholesterol levels upon ingestion, comprising the use of a soy protein product with a further specified minimum content of intact 7S ( $\alpha + \alpha' + \beta$ ) and/or 11S subunits (A + B), a minimum 7S enthalpy peak or a minimum solubility.

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In another aspect of the present invention furthermore provides the products which is the result of said method and the use of said products to lower blood cholesterol levels upon ingestion to obtain a health benefit.

20 In a preferred embodiment of the invention it is the soy protein is incorporated in the food product in the form of a soy protein product.

The food products containing soy protein more specifically are believed facilitate the formation of peptides which has a cholesterol lowering effect.

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According to a currently especially preferred embodiment of the present invention the level of active Trypsin inhibitors in the incorporated soy protein product is less than 20 % of the original activity in the beans, such as e.g. less than 19%, eg. less than 18%, such as less than 17%, eg. less than 16%, such as less than 15%, eg. less than 14%,  
30 such as less than 13%, eg. less than 12%, such as less than 11%, eg. less than 10%, such as less than 9%, eg. less than 8%, such as less than 7%, eg. less than 6%, such as less than 5%.

In one aspect the soy protein products to be incorporated in a food product according  
35 to the invention comprise more than 50% protein of dry matter, such as at least 55%,

e.g. at least 60%, e.g. at least 65%, e.g. at least 70%, such as 75%, e.g. at least 80%, such as at least 85%, e.g. at least 90%, such as at least 95% protein of dry matter.

- 5 The soy protein product to be incorporated in a food product according to the invention is only denatured to a minimal extent i.e. meaning that preferably at least 50%, such as at least 52%, preferably at least 54%, such as at least 56%, preferably at least 58%, such as at least 60%, preferably at least 62%, such as at least 64%, preferably at least 66%, such as at least 68%, preferably at least 70%, such as at least 72%,  
10 preferably at least 74%, such as at least 76%, preferably at least 78%, such as at least 80%, preferably at least 82%, such as at least 84%, preferably at least 86%, such as at least 88%, preferably at least 90%, such as at least 92%, preferably at least 94%, such as at least 96%, preferably at least 98%, such as at least 99%, of the 7S and 11S subunits are preserved in their undenatured form, thereby referring either to the  
15 individual subunits of 7S and 11S alone or all subunits of 7S and 11S combined. It is especially preferred that the globular structure is mainly folded with the hydrophobic part of the molecules inside the globular structure (a disk with room for all fat soluble substances inside the molecule including the isoflavons, which are linked to the hydrophobic side of the proteins by hydrogen bonds).

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- Thus, below part of a general process for the preparation of a soy protein product to be incorporated into a food product according to the invention will be described with respect to a soy flour starting material, although other soy protein and vegetable protein containing starting materials e.g. flakes may also be used. The general  
25 process combines several precipitation steps under conditions which do not denature the globular 11S and 7S proteins. The process allows for two important objectives to be met. Firstly, it allows for removal of TI from the globular soy proteins without applying traditional methods that might denature the globular proteins and complicate the process. Secondly, the process allows for a partial fractionation of the globular  
30 proteins. The first precipitate obtained at modestly acidic pH is thus enriched in 11S globular proteins, whereas the precipitate obtained at more acidic pH is enriched in the 7S globular proteins. It is thus possible to obtain soy protein products with very high amounts of specific globular proteins. Further processing of a fraction from the acidic precipitation which is enriched in 7S $\alpha'$ , by for example size fractionation to remove  
35 molecular species of small size, would therefore provide a convenient method to obtain soy protein comprising very high amounts of the 7S $\alpha'$  subunit. Given the current

focus on the effects of the 7S $\alpha$ ' subunit soy protein products with increased amounts of this subunit may prove to be of particular interest.

To obtain an ISP with a much higher content of 7S - and thus 7S $\alpha$ ' - a cultivar with a natural relation between 7S and 11S of up to 2:1 can be selected and/or further separation steps may be applied. Membrane filtration technology could for example be used to concentrate the 7 S fraction without denaturing the globular structure further. This technology is often called "crossflow" membrane filtration as the liquid is flowing tangential to the membrane surface and thus inhibits formation of deposits. In this case we have globular proteins in the form of ISP obtained by the method according to the invention. This could for example be a product produced as described in example 4 or a fraction precipitated out at pH 3.5 and neutralized afterwards. The proteins in these two ISPs can be separated according to molecular size in three fractions:

- 1) below 100 kDa
- 2) between 100 and 250 kDa
- 3) above 250 kDa

The separation can be carried out in two steps:

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Step 1: Removal of molecules with a molecular weight below 100 kDa and collection of a product fraction with the higher molecular weights (concentrate).

Step 2: Removal of the 11S fraction by applying a membrane with a cut-off at approximately 250 kDa. The product fraction, which is the permeate is enriched in 7S proteins.

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The equipment is typically tubular with a housing of stainless steel. The tubes are equipped with the suitable membrane for the separation. The first step can be an ultrafiltration step with an operating pressure below 10 bars. The second step can be a microfiltration with an operating pressure below 3 bars. (Two different membranes is used in the two membrane filtration steps).

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As the separation has to be performed on a molecular level, the ISP must be completely dissolved in the water phase. The separation is normally carried out at at

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pH 6.5-7 and the ISP is dissolved in water in less than 4 % w/v at from 5-25°C, preferably at from 15 to 20 °C.

5 The permeate, in step 2 above, must be concentrated before spray drying. This is preferably done by reverse osmosis to take out water and concentrate the protein up to a level of 20-40 % dry matter. An alternative is concentration in high vacuum evaporators at low temperature (below 20 °C).

10 The above example of the application of a further purification technique have been included for illustrative purposes only. It will be obvious to the skilled man that other techniques for protein purification that are known in the art may be applied as well.

The amount of intact 7S subunits ( $\alpha + \alpha' + \beta$ ) and 11S subunits (A + B) in soy protein products to be incorporated in a food product according to the invention preferably  
15 constitute more than 5 % of the total soy protein content, such as more than 10 %, for example more than 15 %, , such as more than 20 %, for example more than 25 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for  
20 example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 51 %, such as more than 52 %, for example more than 53 %, such  
25 as more than 54 %, for example more than 55 %, such as more than 56 %, for example more than 57 %, such as more than 58 %, for example more than 59 %, such as more than 60 %, for example more than 61 %, such as more than 62 %, for example more than 63 %, such as more than 64 %, for example more than 65 %, such as more than 66 %, for example more than 67 %, such as more than 68 %, for  
30 example more than 69 %, such as more than 70 %, for example more than 71 %, such as more than 72 %, for example more than 73 %, such as more than 74 %, for example more than 75 %, such as more than 77 %, for example more than 79 %, such as more than 81 %, for example more than 83 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

The amount of intact 7S subunits ( $\alpha + \alpha' + \beta$ ) in soy protein products to be incorporated in a food product according to the invention preferably constitute more than 1 % of the total soy protein content, such as more than 2 %, for example more than 3 %, such as more than 4 %, for example more than 5 %, such as more than 6 %, for example more than 7 %, such as more than 8 %, for example more than 9 %, such as more than 10 %, for example more than 11 %, such as more than 12 %, for example more than 13 %, such as more than 14 %, for example more than 15 %, such as more than 16 %, for example more than 17 %, such as more than 18 %, for example more than 19 %, such as more than 20 %, for example more than 21 %, such as more than 22 %, for example more than 23 %, such as more than 24 %, for example more than 25 %, such as more than 26 %, for example more than 27 %, such as more than 28 %, for example more than 29 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 55 %, such as more than 60 %, for example more than 65 %, such as more than 70 %, for example more than 75 %, such as more than 80 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

The amount of intact 7S subunit  $\alpha$  in soy protein products to be incorporated in a food product according to the invention preferably constitute more than 1 % of the total soy protein content, such as more than 2 %, for example more than 3 %, such as more than 4 %, for example more than 5 %, such as more than 6 %, for example more than 7 %, such as more than 8 %, for example more than 9 %, such as more than 10 %, for example more than 11 %, such as more than 12 %, for example more than 13 %, such as more than 14 %, for example more than 15 %, such as more than 16 %, for example more than 17 %, such as more than 18 %, for example more than 19 %, such as more than 20 %, for example more than 21 %, such as more than 22 %, for example more than 23 %, such as more than 24 %, for example more than 25 %, such as more than 26 %, for example more than 27 %, such as more than 28 %, for example more than 29 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for

example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 55 %, such as more than 60 %, for example more than 65 %, such as more than 70 %, for example more than 75 %, such as more than 80 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

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The amount of intact 7S subunit  $\alpha'$  in soy protein products to be incorporated in a food product according to the invention preferably constitute more than 1 % of the total soy protein content, such as more than 2 %, for example more than 3 %, such as more than 4 %, for example more than 5 %, such as more than 6 %, for example more than 7 %, such as more than 8 %, for example more than 9 %, such as more than 10 %, for example more than 11 %, such as more than 12 %, for example more than 13 %, such as more than 14 %, for example more than 15 %, such as more than 16 %, for example more than 17 %, such as more than 18 %, for example more than 19 %, such as more than 20 %, for example more than 21 %, such as more than 22 %, for example more than 23 %, such as more than 24 %, for example more than 25 %, such as more than 26 %, for example more than 27 %, such as more than 28 %, for example more than 29 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 55 %, such as more than 60 %, for example more than 65 %, such as more than 70 %, for example more than 75 %, such as more than 80 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

The 7S enthalpy peak in a soy protein product to be incorporated in a food product according to the invention preferably constitute at least 0.1 J/g protein, such as 0.2, e.g. 0.3, such as 0.4, e.g. 0.5, such as 0.6, e.g. 0.7, such as 0.8, e.g. 0.9, such as 1.0,

e.g. 1.1, such as 1.2, e.g. 1.3, such as 1.4, e.g. 1.5, such as 1.6, e.g. 1.7, such as 1.8, e.g. 1.9, such as 2.0 e.g. 2.2, such as 2.4, e.g. 2.6, such as 2.8, e.g. 3.0, such as 3.5, e.g. 4.0, such as 4.5, e.g. 5.0, such as 6.0, e.g. 7.0, such as 8.0, e.g. 9.0, such as 10.0, e.g. 11.0, such as at least 12.0 J/g protein.

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In a soy protein product to be incorporated in a food product according to the invention the soluble proteins preferably constitute more than 55 %, such as more than 60%, e.g. more than 65%, such as more than 70%, e.g. more than 75%, such as more than 80%, e.g. more than 82%, such as more than 84%, e.g. more than 86%, such as more than 88%, e.g. more than 90%, such as more than 92%, e.g. more than 94%, such as  
10 more than 95%, e.g. more than 96%, e.g. more than 97%, such as more than 98%, e.g. more than 99%.

Phytoestrogen compounds according to the present invention are defined as naturally  
15 occurring plant substances, said substances being either structurally or functionally similar to 17-estradiol or generating estrogenic effects. Phytoestrogens consist of a number of classes including isoflavones, coumestans, lignans and resorcylic acid lactones. Examples of isoflavones according to the present invention are genistein, daidzein, equol, glycitein, biochanin A, formononetin, and O-desmethylangolesin. The  
20 phytoestrogen compounds of a soy protein product according to the present invention are preferably isoflavones, more preferably genistein, daidzein, glycitein and/or equol, yet more preferably genistein and/or daidzein, and even more preferably genistein. A preferred soy protein product according to the present invention may accordingly comprise a single isoflavone, such as genistein, daidzein, glycitein or equol, or it may  
25 comprise at least one isoflavone selected from the group comprising at least genistein, daidzein, glycitein and equol. When present in the plant the isoflavones are mainly in a glucoside form, i.e. attached to a sugar molecule. This glucoside form can be deconjugated to yield a so-called aglycone form, which is the biologically active species. A soy protein product according to the present invention may comprise  
30 isoflavones in glucoside and/or aglycone forms regardless of whether the deconjugation to the aglycone form has taken place biologically, in vitro or by any other means whereby the isoflavones are included in a soy protein product according to the present invention or if the aglycone forms are the native form of the isoflavones.

35 A soy protein product to be incorporated in a food product according to the invention may optionally also contain phytoestrogens, such as isoflavons. The phytoestrogen

compound is preferably present in an amount, referring to the aglycone forms, of at least about 0.10 weight percent of the soy protein content, such as at least about 0.11 weight percent, for example at least about 0.12 weight percent of the soy protein content, such as at least about 0.14 weight percent, for example at least about 0.16 weight percent, such as at least about 0.18 weight percent, for example at least about 0.20 weight percent, such as at least about 0.22 weight percent, for example at least about 0.24 weight percent, such as at least about 0.25 weight percent, for example more than about 0.25 weight percent, such as at least about 0.26 weight percent, for example at least about 0.28 weight percent, such as at least about 0.30 weight percent, for example at least about 0.32 weight percent, such as at least about 0.33 weight percent, for example more than about 0.33 weight percent, such as at least about 0.35 weight percent, for example at least about 0.40 weight percent, such as at least about 0.45 weight percent, for example at least about 0.50 weight percent, such as at least about 0.55 weight percent, for example at least about 0.60 weight percent, such as at least about 0.65 weight percent, for example at least about 0.70 weight percent, such as at least about 0.75 weight percent, for example at least about 0.80 weight percent, such as at least about 0.85 weight percent, for example at least about 0.90 weight percent, such as at least about 1.0 weight percent of the soy protein content, and preferably less than 2.50 weight percent of the soy protein content.

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A soy protein product according to the invention may optionally have the trypsin inhibitors partly or fully destroyed or removed. The amount of ACTIVE trypsin inhibitor in a soy protein product according to the invention may preferably be less than 50% of the amount in the original soy bean, such as less than 40%, for example less than 30%, such as less than 25%, for example less than 20%, such as less than 15%, for example less than 10%, such as less than 5 %, for example less than 1%.

Researchers have shown that specific amino acids may to some extent effect serum lipid levels and potentially alleviate cardiovascular diseases. Animal studies have indicated that the amino acid lysine increases serum cholesterol levels, while arginine counteracts this effect (Kurowska et al., J. Nutr. 124, 364-370 (1994) and Sanchez et al., Med. Hypotheses 35, 324-329 (1991). This observation appears to be in correspondence with the well established influence of NO on vasodilation, since arginine may potentially be converted to citrullin and NO by NO-synthetase. Thus according to a presently preferred hypothesis soy protein having a high arginine to lysine ratio effects serum lipid levels and alleviates symptoms of cardiovascular



diseases to a greater extent than soy protein having a lower or normal arginine to lysine ratio. Consequently, isolated, potentially processed, soy protein having a high arginine to lysine ratio is a particularly preferred soy protein product according to the present invention. Preferably the soy protein product according to the present invention should have an arginine to lysine ratio of at least about 1.0, such as at least about 1.1, for example at least about 1.2, such as at least about 1.3, for example at least about 1.4, such as at least about 1.5, for example at least about 1.6, such as at least about 1.7, for example at least about 1.8, such as at least about 1.9, for example more than about 2, such as at least about 2.1, for example at least about 2.2, such as at least about 2.5, for example at least about 2.75, such as at least about 3, for example more than about 3.3, such as at least about 3.6, for example at least about 4, such as at least about 4.5, for example at least about 5, such as at least about 6, for example at least about 7, such as at least about 8, for example at least about 9, such as at least about 10, for example at least about 11, such as at least about 12, for example at least about 13, such as at least about 14.

Phytoestrogen compounds according to the present invention are defined as naturally occurring plant substances, said substances being either structurally or functionally similar to 17-estradiol or generating estrogenic effects. Phytoestrogens consist of a number of classes including isoflavones, coumestans, lignans and resorcylic acid lactones. Examples of isoflavones according to the present invention are genistein, daidzein, equol, glycitein, biochanin A, formononetin, and O-desmethylangolesin. The phytoestrogen compounds of a soy protein product according to the present invention are preferably isoflavones, more preferably genistein, daidzein, glycitein and/or equol, yet more preferably genistein and/or daidzein, and even more preferably genistein. A preferred soy protein product according to the present invention may accordingly comprise a single isoflavone, such as genistein, daidzein, glycitein or equol, or it may comprise at least one isoflavone selected from the group comprising at least genistein, daidzein, glycitein and equol. When present in the plant the isoflavones are mainly in a glucoside form, i.e. attached to a sugar molecule. This glucoside form can be deconjugated to yield a so-called aglycone form, which is the biologically active species. A soy protein product according to the present invention may comprise isoflavones in glucoside and/or aglycone forms regardless of whether the deconjugation to the aglycone form has taken place biologically, in vitro or by any other means whereby the isoflavones are included in a soy protein product according to the present invention or if the aglycone forms are the native form of the isoflavones.

The phytoestrogen compound is preferably present in an amount of at least about 0.10 weight percent of the soy protein content. More preferably the phytoestrogen compound is present in an amount of at least 0.10 weight percent of the soy protein content, such as at least about 0.11 weight percent, for example at least about 0.12 weight percent, such as at least about 0.14 weight percent, for example at least about 0.16 weight percent, such as at least about 0.18 weight percent, for example at least about 0.20 weight percent, such as at least about 0.22 weight percent, for example at least about 0.24 weight percent, such as at least about 0.25 weight percent, for example more than about 0.25 weight percent, such as at least about 0.26 weight percent, for example at least about 0.28 weight percent, such as at least about 0.30 weight percent, for example at least about 0.32 weight percent, such as at least about 0.33 weight percent, for example more than about 0.33 weight percent, such as at least about 0.35 weight percent, for example at least about 0.40 weight percent, such as at least about 0.45 weight percent, for example at least about 0.50 weight percent, such as at least about 0.55 weight percent, for example at least about 0.60 weight percent, such as at least about 0.65 weight percent, for example at least about 0.70 weight percent, such as at least about 0.75 weight percent, for example at least about 0.80 weight percent, such as at least about 0.85 weight percent, for example at least about 0.90 weight percent, such as at least about 1.0 weight percent of the soy protein content, and preferably less than 2.50 weight percent of the soy protein content.

The present invention also provides the use of a food product containing soy protein as a functional food effective in preventing, treating, prophylactically treating, alleviating and/or eliminating a cardiovascular disease in a subject. The present invention also provides the use of such a food product containing soy protein as a functional food effective in preventing, treating, prophylactically treating, alleviating and/or eliminating arteriosclerosis or a related cardiovascular disease in a subject. The present invention also provides the use of such a food product containing soy protein as a functional food for treating diabetic subjects, said treatment being effective in lowering serum levels of glucose and/or insulin and/or lipids. The present invention also provides the use of such a food product containing soy protein as a functional food effective in treating and/or alleviating type 2 diabetes, the metabolic syndrome as defined herein and/or cardiovascular diseases associated therewith in a subject. The present invention also provides the use of such a food product containing soy protein as a functional food effective in treating subjects diagnosed as having a pulmonary

disease, said treatment being effective at least in increasing FEV1 of a subject, as measured by forced expiratory volume in the first second of expiration. The present invention also provides the use of such a food product containing soy protein as a functional food effective in treating and/or alleviating pulmonary diseases in a subject.

- 5 The present invention also provides the use of such a food product containing soy protein for use in treating arteriosclerosis or a related cardiovascular disease in a subject. The present invention also provides the use of such a food product containing soy protein for use in the treatment of diabetic subjects, said treatment being particularly effective in lowering serum levels of glucose and lipids in a subject. The
- 10 present invention also provides the use of such a food product containing soy protein for use in the treatment and/or alleviation of a pulmonary disease in a subject, said treatment and/or alleviation resulting in an increased FEV1 of a subject, as measured by forced expiratory volume in the first second of expiration.

- 15 A soy protein product to be incorporated in a food product according to the present invention may optionally comprise a carbohydrate source, flavoring agents, vitamins, minerals, electrolytes, trace elements and other conventional additives. The nutritional soy protein products to be incorporated in a food product according to the present invention may in one embodiment also comprise one or more flavoring agents such as
- 20 cocoa, vanilla, lime, strawberry or soup flavors, such as mushroom, tomato or bouillon and/or sweeteners such as aspartame as well as other additives such as xanthan gum.

- When a carbohydrate source is present in a soy protein product to be incorporated in a
- 25 food product according to the present invention, it is preferably present in an amount of less than 30 weight percent such as less than 25 weight percent of the soy protein product. Preferably, the amount of carbohydrate amounts to at least 5 weight percent, more preferred at least 10 weight percent, and most preferred at least 15 weight percent, of the soy protein product. The preferred carbohydrates for use in a soy
- 30 protein product to be incorporated in a food product according to the present invention are dextrose, fructose and/or maltodextrin, or glucose. Skimmed milk and lecithinated fat reduced cacao are other possible carbohydrate sources. When a soy protein product to be incorporated in a food product according to the present invention is for use in the prevention and/or treatment of type 2 diabetes, the metabolic syndrome and
- 35 associated cardiovascular diseases, lecithinated fat reduced cacao is particularly preferred. Other preferred carbohydrates for use in a soy protein product to be

incorporated in a food product according to the present invention for use in the prevention and/or treatment of type 2 diabetes, the metabolic syndrome and associated cardiovascular diseases are polydextrose or saccharose, but these should be limited using other sweeteners like e.g. aspartame.

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Vitamins and minerals may optionally be added to a soy protein product to be incorporated in a food product according to the present invention in accordance with the limits laid down by health authorities. A soy protein product to be incorporated in a food product according to the present invention may comprise all recommended  
10 vitamins and minerals. The vitamins will typically include A, B1, B2, B12, folic acid, niacin, panthotenic acid, biotin, C, D, E and K. The minerals will typically include iron, zinc, iodine, copper, manganese, chromium and selenium. Electrolytes, such as sodium, potassium and chlorides, trace elements and other conventional additives may also be added in recommended amounts.

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A soy protein product to be incorporated in a food product according to the present invention may be used for special dietary use, preferably for lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides in subjects such as hyperlipidemic patients or normocholesterolemic patients suffering from a  
20 cardiovascular disease, and/or for lowering serum levels of glucose and/or insulin and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or for increasing glucose tolerance and/or insulin sensitivity and/or for preventing, treating and/or alleviating impaired glucose tolerance and/or insulin secretory failure in diabetic subjects and/or for preventing, treating and/or alleviating an arteriosclerotic condition  
25 by reducing the influx of lipoproteins and/or cholesterol and/or triglycerides into the endocelium of the arterial wall of a diabetic subject suffering from a cardiovascular disease. For example, from one to three daily meals of ordinary food can be supplemented or replaced by a soy protein product to be incorporated in a food product according to the present invention. Hereby, significant reductions in serum  
30 levels of cholesterol and/or LDL-cholesterol and/or triglycerides can be obtained, as well as an improvement of serum HDL/LDL-cholesterol ratio and/or an increase in serum HDL-cholesterol levels. The soy protein product may provide from about 50 to about 250 kcal per serving.

35 The daily dose of a soy protein product to be incorporated in a food product according to the present invention may comprise an energy content of from 400 to 800, in

particular from 450 to 800 kcal/day, which is considered to be a very low calorie diet (VLCD), or it may comprise an energy content of from 800 to 1200 kcal/day, which is considered to be a low-calorie diet (LCD). In another medical embodiment of the present invention, the energy content may correspond to the daily energy requirement of a normal person.

The present invention also provides a soy protein product to be incorporated in a food product according to the invention in the form of a micronutrient. In this connection a micronutrient is a nutritional supplement and/or a pharmacological soy protein product and/or a functional food comprising i) a synthetic phytoestrogen-like compound capable of binding to an estrogen receptor or an estrogen-like receptor, and/or ii) a naturally occurring, plant-extractable compound in an amount, on a weight per weight basis, in excess of the amount of said compound, when it is present in a natural host such as a plant cell from which the compound can be extracted or isolated, iii) soy peptides obtainable from a partial hydrolysis of soy protein and iv) soy lecithin.

The naturally occurring, plant-extractable compound is preferably but not limited to compounds capable of binding to an estrogen receptor, an estrogen-like receptor, a beta-2-adrenergic receptor or a receptor belonging to the class of beta-2-adrenergic receptors. When the naturally occurring compounds are isolated from plants such as soybeans, they may be selected from the group at least containing phytoestrogens such as soybean phytoestrogens such as soybean isoflavones, soy protein or fragments thereof, e.g. peptides or amino acid sequences, soybean fibers, lecithin, linolenic acid, an antioxidant, a saponin, a lignan, a protease inhibitor, a trypsin inhibitor, and a tyrosine kinase inhibitor. Additional constituents of the micronutrient may preferably be selected among a DNA topoisomerase inhibitor, a ribosome kinase inhibitor, a growth control factor such as e.g. epidermal growth factor, transforming growth factor alpha, platelet derived growth factor, and preferably any growth control factor controllable by a tyrosine kinase activity. The micronutrient may also comprise ormeloxifene and/or levormeloxifene as described by among others Holm et al. (1997) in Arteriosclerosis, Thrombosis, and Vascular Biology 17 (10), 2264 – 2272, and in Clinical Investigation, 100 (4), 821 – 828. When the naturally occurring compound is an isoflavone, the isoflavone may have been deconjugated to the aglycone form either biologically or in vitro prior to the incorporation in the micronutrient.

In one particularly preferred embodiment the present invention provides a soy protein product or a micronutrient according to the present invention in combination with a functional food ingredient comprising a sterol, preferably an ingredient selected from the group comprising a stanol ester, a tocotrienol, a mevinolin, and a phytosterol compound such as e.g. campesterol, sitosterol or stigmasterol, or a combination thereof.

According to one preferred embodiment, a soy protein product or a micronutrient according to the present invention is for use as a functional food ingredient. A soy protein product or a micronutrient according to the present invention may also be administered as a probe or by intravenous administration, or in tablet or capsule form. The present invention also provides a pharmaceutical preparation comprising the a soy protein product or a micronutrient according to the present invention, use of the a soy protein product or a micronutrient according to the present invention in therapy and/or a diagnostic method performed on the human or animal body, use of a soy protein product or a micronutrient according to the present invention in the manufacture of a functional food, use of a soy protein product or a micronutrient according to the present invention in the manufacture of a functional food for treating a subject suffering from cardiovascular diseases, use of a soy protein product or a micronutrient according to the present invention in the manufacture of a functional food for treating a subject suffering from type 2 diabetes, the metabolic syndrome and/or cardiovascular diseases associated therewith in a diabetic subject and use of a soy protein product or a micronutrient according to the present invention in the manufacture of a functional food for treating a subject suffering from pulmonary diseases.

The micronutrient is particularly useful in preventing, treating, prophylactically treating and/or alleviating hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis and/or related cardiovascular diseases and in preventing and/or treating type 2 diabetes, the metabolic syndrome and/or cardiovascular diseases associated therewith in a diabetic subject and in preventing, treating, prophylactically treating and/or alleviating a pulmonary disease such as e.g. a disease selected from the group comprising inflammation of the airways, bronchoconstriction, bronchitis, asthma, and small airways diseases.

In one embodiment the present invention provides a soy protein product to be incorporated in a food product according to the present invention for use as a functional food or as a dietary preparation. A soy protein product to be incorporated in a food product according to the present invention for use as a functional food or as a dietary preparation may preferably be used in preventing, treating, prophylactically treating and/or alleviating cardiovascular diseases such as e.g. a disease selected from the group comprising hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, myocardial infarction, and hypertension, in a subject, preferably for use in preventing, treating, prophylactically treating and/or alleviating arteriosclerosis and/or atherosclerosis in a subject. A soy protein product to be incorporated in a food product according to the present invention for use as a functional food or as a dietary preparation may also preferably be used in preventing, treating, alleviating and/or eliminating type 2 diabetes. A soy protein product to be incorporated in a food product according to the present invention for use as a functional food or as a dietary preparation may also preferably be used in preventing, treating, alleviating and/or eliminating a cardiovascular disease, such as e.g. hypercholesterolemia, hypertriglyceridemia, hypertension, hyperglycemia, hyperinsulinemia, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, and myocardial infarction, in a diabetic subject. A soy protein product to be incorporated in a food product according to the present invention for use as a functional food or as a dietary preparation may also preferably be used for preventing and/or treating pulmonary diseases, such as preferably a disease selected from the group comprising inflammation of the airways, bronchoconstriction, bronchitis, asthma, and small airways diseases, in a subject.

The present invention also provides the use of a soy protein product to be incorporated in a food product according to the present invention as a functional food and/or in the manufacture of a functional food for preventing, treating, prophylactically treating and/or alleviating cardiovascular diseases such as e.g. a disease selected from the group comprising hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, myocardial infarction, and hypertension, particularly a disease selected from the group comprising arteriosclerosis and atherosclerosis, in a subject. The present invention also provides the use of a soy protein product to be incorporated in a food product according to the present invention

as a functional food and/or in the manufacture of a functional food for preventing, treating and/or alleviating type 2 diabetes and/or the metabolic syndrome in a subject and/or a cardiovascular disease in a diabetic subject. The present invention also provides the use of a soy protein product to be incorporated in a food product  
5 according to the present invention as a functional food and/or in the manufacture of a functional food for preventing, treating, prophylactically treating and/or alleviating pulmonary diseases such as e.g. a disease selected from the group comprising inflammation of the airways, bronchoconstriction, bronchitis, asthma, and small airways diseases, in a subject.

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The food product containing soy protein according to the present invention is effective in lowering levels of cholesterol in normocholesterolemic patients by at least 2%, for example at least 5%, such as at least 8%, for example at least 10%, such as at least 12%, for example at least 14%, such as at least 16%, for example at least 18%, such  
15 as at least 20%, for example at least 25%, such as at least 30%. The food product containing soy protein according to the present invention is effective in lowering levels of triglycerides in normocholesterolemic patients by at least 10%, such as at least 12%, for example at least 14%, such as at least 16%, for example at least 18%, such as at least 20%, for example at least 25%, such as at least 30%.

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The food product containing soy protein according to the present invention is effective in lowering levels of cholesterol in mildly hypercholesterolemic patients by at least 3%, for example at least 5%, such as at least 8%, for example at least 10%, such as at least 12%, for example at least 15%, such as at least 20%, for example at least 25%,  
25 such as at least 30%, for example at least 35%, such as at least 40%, for example at least 45%. The food product containing soy protein according to the present invention is effective in lowering levels of triglycerides in mildly hypercholesterolemic patients by at least 15%, such as at least 20%, for example at least 25%, such as at least 30%, for example at least 35%, such as at least 40%, for example at least 45%.

30

The food product containing soy protein according to the present invention is effective in lowering levels of cholesterol in severely hypercholesterolemic patients by at least 3%, for example at least 5%, such as at least 8%, for example at least 10%, such as at least 12%, for example at least 15%, such as at least 20%, for example at least 25%,  
35 such as at least 30%, for example at least 35%, such as at least 40%, for example at least 45%, such as at least 50%, for example at least 55%, such as at least 60%. The



food product containing soy protein according to the present invention is effective in lowering levels of triglycerides in severely hypercholesterolemic patients by at least 20%, for example at least 25%, such as at least 30%, for example at least 35%, such as at least 40%, for example at least 45%, such as at least 50%, for example at least 55%, such as at least 60%.

A food product containing soy protein according to the present invention for use as a functional food and/or the use of a food product containing soy protein according to the present invention as a functional food and/or in the manufacture of a functional food for preventing, treating, prophylactically treating and/or alleviating cardiovascular diseases in a subject may be effective in reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or causing dilation of blood vessels and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or lowering serum levels of total cholesterol and/or LDL-cholesterol and/or homocystein and/or triglycerides and/or increasing the serum HDL/LDL-cholesterol ratio and/or increasing serum levels of HDL-cholesterol in a subject and/or preventing, reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or reducing or eliminating the risk of a subject contracting angina pectoris and/or reducing or eliminating the risk of a subject contracting a myocardial infarction.

A food product containing soy protein according to the present invention for use as a functional food and/or the use of a food product containing soy protein according to the present invention as a functional food and/or in the manufacture of a functional food for preventing and/or treating diabetes and/or the metabolic syndrome and/or a cardiovascular disease associated therewith in a subject may be effective in i) lowering serum glucose levels and/or ii) reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or causing dilation of blood vessels and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or iii) lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels and/or iv) increasing glucose tolerance and/or insulin sensitivity and/or v) alleviating impaired glucose tolerance and/or insulin secretory failure and/or improving insulin secretion and/or vi) preventing, treating, alleviating, and/or eliminating cardiovascular diseases, such as e.g. hypercholesterolemia, hypertriglyceridemia,

other hyperlipidemias, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, myocardial infarction, hypertension, hyperglycemia, and hyperinsulinemia, in a diabetic subject and/or preventing, reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or reducing or eliminating the risk of a diabetic subject contracting angina pectoris and/or reducing or eliminating the risk of a diabetic subject contracting a myocardial infarction and/or in treating a procoagulant state and/or an increased activity of clotting factors, insulin resistance, glycosidation and/or oxidation and/or chemical modification of lipoproteins, as well as impaired glucose tolerance.

A food product containing soy protein according to the present invention for use as a functional food and/or the use of a food product containing soy protein according to the present invention as a functional food and/or in the manufacture of a functional food for preventing, treating, prophylactically treating and/or alleviating pulmonary diseases may be effective in i) preventing, treating, prophylactically treating and/or alleviating asthma and/or ii) reducing and/or eliminating mucus hypersecretion and/or dyspnea in a subject suffering from asthma and/or iii) increasing FEV1 of a subject as measured by forced expiratory volume in the first second of expiration and/or iv) preventing, treating, prophylactically treating, alleviating and/or reducing inflammation of the airways and/or v) preventing, treating, prophylactically treating and/or alleviating bronchoconstriction.

In another embodiment the present invention provides the use of a food product containing soy protein according to the present invention in the treatment of cardiovascular diseases in the human or animal body in an amount effective in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or preventing, reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or reducing or eliminating the risk of a subject contracting angina pectoris and/or reducing or eliminating the risk of a subject contracting a myocardial infarction, and/or alleviating the clinical condition of patients contracting a myocardial infection. The

cardiovascular disease is preferably a cardiovascular disease selected from the group comprising hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, myocardial infarction, and hypertension and more preferred  
5 selected from arteriosclerosis and atherosclerosis.

In another embodiment the present invention provides the use of a food product containing soy protein according to the present invention in the treatment of type 2 diabetes and/or the metabolic syndrome in an amount effective in lowering serum  
10 levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or glucose and/or increasing serum levels of HDL-cholesterol and/or homocystein and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or improving glucose tolerance and/or increasing insulin sensitivity and/or alleviating  
15 impaired glucose tolerance and/or improving insulin secretion and/or reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or preventing, reducing or eliminating the risk of a subject contracting angina pectoris and/or preventing, reducing or eliminating the risk of a subject  
20 contracting a myocardial infarction and/or preventing, treating, prophylactically treating, alleviating and/or eliminating hypertension and/or hyperglycemia and/or hyperinsulinemia and/or hypercholesterolemia and/or hypertriglyceridemia and/or arteriosclerosis and/or atherosclerosis and/or arteriolosclerosis in a diabetic subject.

25 In another embodiment the present invention provides the use of a food product containing soy protein according to the present invention in the treatment of a pulmonary disease in a human or animal body, preferably a disease selected from the group comprising inflammation of the airways, bronchoconstriction, bronchitis, asthma, and small airways diseases, in an amount effective in preventing, treating,  
30 prophylactically treating and/or alleviating inflammation of the airways and/or bronchoconstriction and/or bronchitis and/or small airways diseases and/or asthma and/or reducing and/or eliminating mucus hypersecretion and/or dyspnea in a subject suffering from asthma and/or increasing FEV1 of a subject as measured by forced expiratory volume in the first second of expiration.

The present invention also provides a method of preventing, treating, prophylactically treating and/or alleviating by therapy a cardiovascular disease in the human or animal body such as an arteriosclerotic condition of a human or animal body, said method comprising administration of a food product containing soy protein according to the present invention in an amount effective in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or preventing, reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or reducing or eliminating the risk of a subject contracting angina pectoris and/or reducing or eliminating the risk of a subject contracting a myocardial infarction, and/or alleviating the clinical condition of patients contracting a myocardial infection. The cardiovascular disease is preferably a cardiovascular disease selected from the group comprising hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, myocardial infarction, and hypertension and more preferred selected from arteriosclerosis and atherosclerosis.

20

The present invention also provides a method of preventing and/or treating by therapy type 2 diabetes and/or the metabolic syndrome in a human or animal body, said method comprising administration to said human or animal body of a food product containing soy protein according to the present invention in an amount effective in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or glucose and/or increasing serum levels of HDL-cholesterol and/or homocystein and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or improving glucose tolerance and/or increasing insulin sensitivity and/or alleviating impaired glucose tolerance and/or improving insulin secretion and/or reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or preventing, reducing or eliminating the risk of a subject contracting angina pectoris and/or preventing, reducing or eliminating the risk of a subject contracting a myocardial infarction and/or preventing, treating, prophylactically treating, alleviating and/or eliminating hypertension and/or hyperglycemia and/or

hyperinsulinemia and/or hypercholesterolemia and/or hypertriglyceridemia and/or arteriosclerosis and/or atherosclerosis and/or arteriolosclerosis in a diabetic subject.

The present invention also provides a method of preventing, treating, prophylactically  
5 treating and/or alleviating by therapy a pulmonary disease in a human or animal body,  
preferably a disease selected from the group comprising inflammation of the airways,  
bronchoconstriction, bronchitis, asthma, and small airways diseases, said method  
comprising administration to said human or animal body of a food product containing  
10 soy protein according to the present invention in an amount effective in preventing,  
treating, prophylactically treating and/or alleviating inflammation of the airways and/or  
bronchoconstriction and/or bronchitis and/or asthma and/or small airways diseases  
and/or reducing and/or eliminating mucus hypersecretion and/or dyspnea in a subject  
suffering from asthma and/or increasing FEV1 of a subject as measured by forced  
expiratory volume in the first second of expiration.

15

The period of treatment is preferably in the range of from 1 to 12 months or more, such  
as from 2 weeks to 9 months, for example from 3 weeks to 6 months, such as from 4  
weeks to 4 months, such as from 6 weeks to 3 months. However, the period of  
treatment shall not be limited to these periods and may e.g. be longer than 12 months,  
20 such as e.g. a lifelong treatment in order to prevent cardiovascular diseases or in order  
to prevent and/or alleviate type 2 diabetes and/or a cardiovascular disease in  
connection therewith or in order to prevent pulmonary diseases.

In one embodiment the present invention provides a pharmaceutical preparation  
25 comprising a food product containing soy protein according to the present invention.  
The pharmaceutical preparation can be prepared in any way known to the skilled  
person.

In another embodiment the present invention provides the use of a food product  
30 containing soy protein according to the present invention as a nutritional preparation  
and/or in the manufacture of a nutritional preparation for lowering serum levels of  
glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or  
homocystein and/or increasing the serum HDL/LDL-cholesterol ratio and/or serum  
levels of HDL-cholesterol in a subject, including a diabetic subject, and/or for  
35 alleviating a pulmonary condition such as e.g. asthma. The nutritional preparation may  
take any form, which is suitable for human or animal consumption. In one preferred

embodiment, the food product containing soy protein is a powdery mixture, which is suspendable, dispersible or emulsifiable in a liquid for human or animal consumption. The liquid is preferably a water-containing liquid such as e.g. water, coffee, tea or juice, including fruit juice. For such a purpose, the food product containing soy protein  
5 may be packed in a package intended for covering part of or the total nutritional requirement for a defined period of time, such as a period of e.g. three days or a week. The present invention also provides the nutritional preparation in the form of a dietary supplement.

- 10 The nutritional preparation according to the present invention is preferably prepared under conditions which minimise the denaturation of the soy proteins.

The soy protein may optionally be combined with soy fiber and soy lecithin, and incorporated into food products by e.g. mixing in blenders at room temperature or in  
15 the form of small beads.

In liquid products the soy protein or blends with other soy components as described above is added as powder blend.

- In many other foods the most preferred form will be as small beads, which will change  
20 the flavour and texture only to small degree and thus make the product much more palatable.

- The undenatured soy protein or blends with other soy components as described above, may be prepared as granulates by traditional granulation processes; in  
25 which water is used as granulation liquid and at temperatures during the granulation step below 70°C at a humidity of the granulate < 90%, below 100°C at a humidity < (see the temperature/humidity relation for 7S), below 130°C at a humidity < 30 %.
- Applicable granulation technologies comprise fluid bed granulation, rapid mixer granulation, open bowl granulation where the water could be added as nozzle spray or  
30 in jet from or in portions. A preferred granulation technology is screw mixers/extruders as the granulate can easily be made as small, even bullet formed particles of chosen size from 200 to 10000 microns at conditions of approx 30 % humidity and at temperatures of below 100°C, e.g. below 90°C, such as below 80°C, e.g. below 70°C, such as below 60°C, e.g. below 50°C, such as below 40°C, e.g. below 30°C. Excess  
35 water in the granulate is normally dried to below 20 %, preferably down to below 10 %, preferably down to below 5 % at temperatures at below 120°C, or below 80°C or

preferably below 60°C by air and/or in vacuum. The resulting granulate has a bulk density of more than 100 up to 1300 gram per liter

The nutritional preparation in one embodiment of the present invention is preferably a functional food or drink, i.e. a readily obtainable edible or drinkable substance that constitutes a food product containing soy protein according to the present invention to provide a medical or pharmaceutical effect. Accordingly, the present invention provides a food product containing soy protein according to the present invention for use as a functional food. Functional foods and drinks are preferably selected from the group comprising dairy products, such as yogurt and yogurt ice cream, juice, such as orange juice or tomato juice, ready made liquids for drinking, a spreadable product such as e.g. a margarine or a vegetable or plant extracted oil, a cereal product, such as a traditional breakfast cereal product, nutritional bars, biscuits, bread, soups, such as tomato soup, a meat product, such as a hamburger, a meat substitute product, and a vegetable product. In a further embodiment, a nutritional preparation according to the present invention may be in the form of a ready made liquid or in a powder form or in the form of a troche, a solid food product containing soy protein such as a nutritional bar, a fruit bar, a cookie, a cake, a bread or a muffin.

In another embodiment, a food product containing soy protein according to the present invention is an acid beverage. This may be prepared from un-denatured soy protein in the form of a soy milk (made from ISP or prepared from soy flour) which is mixed with soy fiber, soy lecithin and pectin before it is added to acid fruit juices. Preferred concentration is 2-5 % calculated as soy protein. The pH is adjusted to approximately 4. The liquid product is quickly heated to 90 °C (pasteurization) and cooled down again. By plate heat exchanger and steam injection, followed by vacuum (approx 0.5 bar) and heat exchanger, the whole process should not take less than 2 minutes, maximum 2 seconds from 70-90-70 °C, which is the critical heating step. The pasteurized product is then aseptic filled on TetraPak containers.

30

In another embodiment, a food product containing soy protein according to the present invention is a liquid nutritional preparation in a water-containing liquid, in which the solid ingredients are suspended, dispersed or emulgated in an amount of from 10 to 25 weight percent. When the liquid nutritional preparation is intended for drinking, it will usually comprise a flavoring agent as discussed above. However, the liquid nutritional preparation may also be used for probe administration.

35

In another embodiment, the present invention relates to the use of a food product containing soy protein according to the present invention as a partial or total diet for an overweight subject, an overweight subject suffering from an arteriosclerotic condition or an overweight subject suffering from a diabetic condition. Obesity is believed to be one of the major causes of diabetes, including type 2 diabetes. Overweight subjects, including overweight diabetic subjects, often have increased serum cholesterol levels and increased triglyceride levels and are therefore more likely to develop cardiovascular diseases. However, the present invention is not limited to treating subjects with an increased risk of contracting a cardiovascular disease, i.e. subjects likely to have increased serum levels of cholesterol and/or triglycerides, or to treating obese diabetic subjects with an increased risk of contracting a cardiovascular disease, i.e. obese diabetic subjects likely to have increased serum levels of cholesterol and/or triglycerides. A food product containing soy protein according to the present invention also has substantial serum cholesterol, serum LDL-cholesterol and serum triglyceride lowering effects in subjects having a more normal lipid profile and in diabetic subjects that do not also suffer from overweight. The medical use of a food product containing soy protein according to the present invention is not limited to overweight or obese subjects, including diabetic subjects, but may be used for normal weight subjects having increased serum levels of cholesterol and/or LDL-cholesterol and/or triglycerides or for subjects with a cardiovascular condition such as e.g. arteriosclerosis or a related condition who have normal serum levels of cholesterol and/or LDL-cholesterol and/or triglycerides. Such increased serum levels of cholesterol and/or LDL-cholesterol and/or triglycerides may be caused by intake of a diet rich in fats or it may be genetically related.

For the purpose of the present invention, subjects having an initial total serum cholesterol level of 5.7 mmol/l or below are considered to have a normal or hypocholesterolemic level, whereas subjects having a total serum cholesterol level above 5.7 mmol/l are considered to be hypercholesterolemic. Accordingly, by treating normocholesterolemic subjects, it is possible to prevent the development of cardiovascular diseases arising from serum cholesterol levels below a concentration of 5.7 mmol/l in subjects, including diabetic subjects, particularly sensitive to developing e.g. arteriosclerosis, or prevent further development of cardiovascular diseases in patients, including diabetic patients, with previous cardiovascular events.



By treating hypercholesterolemic subjects, it is possible to prevent the development of cardiovascular diseases arising from serum cholesterol levels above a concentration of 5.7 mmol/l in subjects sensitive to developing e.g. arteriosclerosis under such conditions.

5

More particularly, subjects having a total serum cholesterol level of from 5.7 mmol/l to 7.9 mmol/l are considered to be mildly hypercholesterolemic. Accordingly, by treating these hypercholesterolemic subjects, it is possible to prevent the development of cardiovascular diseases arising from serum cholesterol levels of from 5.7 to 7.9  
10 mmol/l. Subjects having a total serum cholesterol level of more than 7.9 mmol/l are considered to be severely hypercholesterolemic. Accordingly, by treating these hypercholesterolemic subjects, it is possible to prevent the development of cardiovascular diseases arising from serum cholesterol levels of more than 7.9 mmol/l.

15 It has also been shown that a food product containing soy protein according to this invention has a potentiating effect to the effect of medications such as e.g. statins and/or niacin. By combining a food product containing soy protein according to the present invention with e.g. statins, such as HMG-CoA-reductase-inhibitors, niacin, bile acid resins, fibrates, nicotinic acid derivatives, oat products, such as oat meal, rye  
20 products, such as rye meal and various fish oil concentrates with a high content of  $\omega$ -3-fatty acids, it is possible to achieve a further 5 to 15% reduction in serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides. The present invention also provides a food product containing soy protein according to the present invention in combination with a statin, preferably an HMG-CoA-reductase-inhibitor, niacin, bile  
25 acid resins, fibrates, oat products, rye products, nicotinic acid derivatives and various fish oil concentrates with a high content of omega-3-fatty acids.

**EXAMPLE 1**

**Preparation of a soy protein product with a high content of intact 7S ( $\alpha + \alpha' + \beta$ ) and/or 11S subunits (A + B)**

- 5 Starting material: G.M.O. free Canadian Soybeans

**Methods:**

- Beans were dehulled and ground. Coarse powder was extracted three times with petroleum spirit (1:4 ratio). Ground with solid carbon dioxide to pass through a 0.5mm  
10 sieve. The flour was suspended in a 100mM Tris-HCl buffer of pH 8.0 in a 1:10 ratio (w/v) and stirred for 1 h at room temperature. Centrifuged (30 min: 9000Xg; 10°C) and supernatant was brought to pH 4.8 with 2 M HCl to induce precipitation. After 2 h at 4°C the dispersion was centrifuged (30 min; 9000Xg; 10°C). The precipitate obtained was washed twice with 10 mM sodium acetate buffer at pH 4.8 in a 1:8 ratio (W/v) and  
15 freeze-dried.

**EXAMPLE 2**

**Preparation of a soy protein product with a high content of intact 7S ( $\alpha + \alpha' + \beta$ ) and/or 11S subunits (A + B)**

5

After dehulling the activity of the Trypsin inhibitors, Bowman-Birk inhibitor and Kunitz Trypsin inhibitor will be reduced by subjecting the dehulled beans to almost boiling water for less than 15 minutes. In order to obtain a final isolate with a level of active Trypsin inhibitors between 5-20 % of the original activity in the beans, a combination with the NADP/thioredoxin system, can be used (from *Escherichia coli* or wheat germ) (described by Jin-an Jiao et al, J. Agric.Food Chem, 40:2333-2336). An alternative is to combine the initial heat treatment with small quantities of Sodium Sulphite ( $\text{Na}_2\text{SO}_3$ ). After crushing and pressing, the rest of the oil will be removed by the traditional treatment with hexane. The soy beans, preferably in the form of flakes will be added to water in an amount less than 15 % w/v. An amount of a combination of sodium and calcium chloride is dissolved in the water in order to make a solution of no more than 0.1 molar. This will increase the denaturation temperatures of both 7S and 11S part of the soy protein. The extraction will be carried out with gentle mixing. The pH is adjusted up to 5.5-7.5 and the temperature should be maximum 68 °C, preferable lower. The time will be restricted to maximum 45 minutes. The un-dissolved material from the beans will be removed by filtration or by centrifugation. The pH is reduced to 4.8-5.5 and the solution is slowly cooled to 5-10 °C during a 1.5 -2 hours period. The solute is removed from the curd. Carbohydrates will be removed by washing once with water. The curd can now be dried carefully by different drying technologies e.g. spray drying/ air temperature below 120 °C. The end temperature of the product should not exceed 60 °C regardless of which drying method is applied.

30

35

**EXAMPLE 3****Preparation of ISP by precipitation at pH 5.4 and/or 3.5**

- 5 Preparations of ISP's from commercial soy flour were carried out as outlined in table 1.

Table 1: Separation scheme for isolates

Step	Action	Fractions	Time, min
1	Disperse material (20g) in water (200ml) at pH 8	-	
2	Stand at ambient	-	60
3	Centrifuge	-	30
4	Separate supernatant and precipitate at pH 5.4 at 4°C or 20°C	-	90-210
	Centrifuge and recover solids IS1	IS1	30
5	Wash 1 <sup>st</sup> solids (S1) with water at pH 8 (100ml)		30
	Centrifuge and recover solids (S2)		30
6	Precipate the supernatant at pH 5.4 (IS2) at 4°C	IS2	90
7	Precipitate the supernatant at pH 3.5 at 4°C for 2 h	IS3	60
8	Dry all materials in freeze drier - 1-2 days		

- 10 Three similar procedures were carried out in which the first precipitation of the isolate at pH 5.4 were varied with respect to time and temperature according to Table 2.

Table 2: Time/temperature variations

Procedure	Time	Temperature, °C
1	3.5	20
2	3.0	4
3	1.5	4

The Flow-sheet for Procedure 3 is shown in figure 1.

- 15 In the full process 7 materials are produced, the washed residue called Fibre, pure isolates, IS1& IS2 and TI-rich isolate IS3& IS4 and soluble solids SS1& SS2.

The samples were examined by chemical analysis for protein, fat and fibre, Trypsin Inhibitor (TI) analysis, Differential Scanning Calorimetry (DSC) and Polyacrylamide electrophoresis (PAGE).

#### Analysis of fractions for protein

- 5 Only small differences in recovery of fractions and proteins were found between the three procedures. The precipitated protein fractions IS1-3 contained 60-65% of the total protein from the flour.

#### Analysis for Trypsin Inhibitor

- 10 TI analysis is International standard for soya products. It is expressed in mg of Bovine trypsin (such as Merck No. 24579) inhibited /mass in g of test sample. TIU trypsin inhibitor units (TIU/mg of sample) 1.9 TIU equals 1 mg of trypsin inhibited.

- The separation according to procedure 3 gave a recovery of 60-62% of the protein in fractions IS 1-3 with only 13% of the original TI. In comparison, gave procedures 1 and 15 2 higher values of respectively 29% and 25% TI in the IS 1-3 fractions. It is therefore clear that procedure 3 offers the best separation of the globular soy proteins from TI.

#### DSC analysis

- The DSC measures the heat of denaturation in proteins as they are heated over a regime from 25 to 180°C. The DSC results showed that the isolate formed at pH 5.4 20 were recovered from soya flour without causing any further denaturation to the proteins. It was furthermore found that the 7 and 11S proteins were partially fractionated by the precipitations, with a relatively higher yield of 11S at pH 5.4 and of 7S at pH 3.5.

- 25 DSC measurements of fractions from procedure 3.

Samples	7S enthalpy peak J/g protein	11S enthalpy peak J/g protein
IS1/procedure 1	0.09	11.51
IS2/procedure 1	0.58	4.87
IS3/procedure 1	3.55	0
IS1/procedure 2	0.3	10.9
IS2/procedure 2	0.23	4.92
IS3/procedure 2	4.36	0.06

IS1/procedure 3	0.65	10.62
IS2/procedure 3	0.47	5.39
IS3/procedure 3	3.78	0.04

#### Analysis of protein composition by SDS PAGE

The 7 fractions from each process were run at an equivalent protein level on a separate gel with standards for molecular size. All the tracks on the gels were stained with a blue dye and also analysed by densitometry. This technique is very sensitive and reveal traces of 7 and 11 S globulins that can not be measured by DSC.

The 11S bands were stronger in the IS1 and IS2 fractions and the 7S in the IS3 fraction, in agreement with the DSC findings.

- 10 The protein pattern for all sample fractions were quite similar for all three gels. The values for the relevant peaks obtained from the densitometry analysis was used for a quantitative analysis of the distribution of the globular soy proteins in the different fractions. The results are summarised in the following table. Two commercial soy protein isolates Suprosoy and Samprosoy are included for comparison:

15

Product/ preparation	Content of 7S alpha	Content of 7S alpha'	Content of 7S	Content of 7S+11S
Suprosoy	8,6	7,4	26,9	61,2
Samprosoy	9,1	7,7	30,3	59,3
Procedure 1 first precipitation at pH 5.4 (ISP1)	8,6	6,5	29,4	64,6
Procedure 1 second precipitation at pH 5.4 (ISP2)	11,0	7,3	32,0	61,6

Procedure 1 precipitation at pH 3.5 (ISP3)	16.7	9.6	39.2	48.8
Procedure 2 first precipitation at pH 5.4 (ISP1)	9,4	7,0	30,6	65,2
Procedure 2 second precipitation at pH 5.4 (ISP2)	12,0	8,2	34,1	62,9
Procedure 2 precipitation at pH 3.5 (ISP3)	15.4	8.5	34.5	45.5
Procedure 3 first precipitation at pH 5.4 (ISP1)	8,9	6,8	29,3	63,0
Procedure 3 second precipitation at pH 5.4 (ISP2)	11,2	7,6	32,2	60,0
Procedure 3 precipitation at pH 3.5 (ISP3)	15.7	8.8	34.0	46.0

#### EXAMPLE 4

##### Preparation of beads/pellets under non-denaturing conditions

Abacor was used for the production of the beads in a twin-screw mixer with a low addition of carob gum to make the beads a bit less water soluble below temperatures of 60°C. The product temperature will be maximum approx 60°C at any point in the mixer. Barrel temperatures was 20°C in all zones.

The extrudate gave a plug flow with little or no die swell so the length could be estimated from the flow rate and the cross-sectional area of the holes in the die. The samples were dried at 60°C for 4h. in a two rack Mitchel tray dryer (Mitchel Dryers Ltd, Carlisle, UK) to a moisture content of 8-9% and stored at ambient temperature in polythene bags. The pellets were dense with values of 530-570 g/l.

#### EXAMPLE 5

##### Preparation of bread with non-denatured soy protein beads

Abacor pellets produced under non-denaturing conditions as in the example above were mixed in CBP doughs according to the table below, to 39kJ/kg over 3-4 min., rested for 6min. moulded and proved at 43°C, 80% relative humidity to a height 100mm over about 35min. The resulting dough gave slightly soft dough initially that processed well later. The loaves were baked for 25 min at 244°C in a reel oven. At the two levels tested 15 and 30% of flour weight, the oven spring was reduced compared to a control of the normal flour control but was still good for recipes with 7.5% gluten in a 30% bead addition or with 5% gluten for a 15% bead addition.

Table 3. Dough recipes

Ingredient	Mass, g	Mass, g	Mass, g
Flour	1000	1000	1000
Water	607	607	607
Fungal amylase	1.4	1.4	1.4
Yeast	42	42	42



Salt	32	32	32
Fat	20	20	20
Gluten	50	75	50
Ascorbic acid	0.1	0.1	0.1
SSL	3	3	3
Abacor beads	300	300	150
Gluten water	75	112.5	75
Bead water	150	150	75

Table 4: Bread volume with beads

	Abacor	Gluten	Spring, %	Volume	Specific volume
Control	0	0	47	1634	4.3
1	15	5	44	1556	4.0
2	15	5	45	1569	4.0
3	30	5	23	1349	3.4
4	30	7.5	30	1424	3.6
5	30	5	23	1348	3.4
6	30	7.5	32	1385	3.5

- 5 During the baking process the temperature of the dough and thus the soy protein, will be below 110°C for approx 90 % of the protein as only the crust temperature will be higher than denaturing temperature of the protein at this humidity level. The same is the case for cookies, crackers and bars, including baked bars.

**EXAMPLE 6**

Preparation of cold-pressed bars with non-denatured soy protein beads

Ingredient	Quantity (g)
Brown sugar	436
Water	100
Citric acid	1.5

5

Heat gently together, stirring continuously, to dissolve sugar, then boil to 130°C.

***Complete Mix***

Ingredient	Quantity (g)
Abacor beads	25
Expanded beads	10
Torrified wheat flakes	25
Puffed rice	15
Rolled oats	15
Choc chips	25
*Orange flavour	1.5
Syrup 4 (hot)	49

10

Allow syrup to cool slightly after boiling then stir in orange flavour.

Blend all together, then press 35g portions into cake bar moulds. Leave to set.

15 Abacor content (as is basis).....15.1%

Soy protein.....10 %

Maximum soy protein content possible:....12.5 %

**EXAMPLE 7****Preparation of baked bars with non-denatured soy protein beads**

5

**Binding mix**

Ingredient	Quantity (g)
Biscuit flour	200
Cake Margarine	250
Soft brown sugar	300
Skimmed milk powder	17
Baking powder	7
Water	100

**Inclusions**

10

Ingredient	Quantity (g)
Abacor beads	265
Rolled oats	200
Abacor expanded beads	100
Dried apricots	200
Bran flakes	200
Currants	100
Raspberry flavour (optional)	5

5

## Procedure:

- Blend all binding mix ingredients in a Hobart mixer for 2 min. on slow speed.
  - Scrape down. Mix for 2 min, on fast speed.
  - Blend in inclusions for 2 min. on slow speed.
- 10
- Spread 535g of this mixture onto a baking tray
  - Spread 100g of bake stable apple jam/filling over this
  - Spread a further 550g of mixture on top.
  - Bake for 18 to 20 min. at 170°C
  - When cool, cut into bars of required size

15

Abacor content 19.4%

Soy protein content 12.8 %

## Evaluation:

- 20 Maximum soy protein content possible..... 13.5 %

**EXAMPLE 8****Preparation of digestive bisquits with non-denatured soy protein beads****Hydrated Abacor beads**

5

Ingredient	Quantity (g)
Water	150
Caster sugar	83
Abacor beads	300

Dissolve sugar in the water then add the Abacor beads. Stir in lightly and leave to stand for 1 hour, turning carefully from time to time.

**Whole product**

10

Ingredient	Quantity (g)
Hydrated Abacor beads*	533
Biscuit flour	780
Wholemeal flour	220
Vegetable shortening	310
Demerara sugar	184
Golden syrup	50
Malt extract	12
Sodium bicarbonate	15.6
Salt	11
Tartaric acid	6.7
Ammonium bicarbonate	3.8
Water	115

Mix all ingredients, except for the flours and the Abacor beads, in a Hobart on speed 1 for 30 sec. Scrape down. Mix on speed 3 for 3 min.

- 5 Add the remaining ingredients and mix on speed 1 for 20 sec., scrape down and mix for a further 40 sec. on speed 1. Rest dough for 15 min. then process using a rotary moulder.

Bake for 7 min at 185°C.

- 10 Abacor content (as is basis).....15.6%  
Soy protein content.....10 %

Evaluation:

Maximum soy protein content possible..... 12.5 %

**EXAMPLE 9****Preparation of chocolate milk with Abacor or placebo with subsequent treatment at very high temperature**

5

The Verum trial substance (Abacor, reg. trade mark, see table below) or the placebo trial substance (see table below) was stirred into long-life chocolate milk (defatted milk, fat content 0.2%). The resulting chocolate milk preparations contain 25 g of soy protein respectively casein per liter.

10

The treatment at very high temperature was performed as follows: The chocolate milk preparations were preheated to 80°C by a plate heat exchanger for approximately 1-2 minutes. The preheated preparations were then treated at very high temperature by direct steam injection to 142°C and kept at this temperature for 4-8 seconds. Following cooling by vacuum to approximately 80°C the preparations were homogenized at 30 bar and subsequently at 150 bar for a maximum of 30 seconds followed by cooling to approximately 20°C over 60-90 seconds.

15

**Table 5.**

20

Content	Amount in 100 g Verum substance (Abacor Reg.)	Amount in placebo substance
Total protein	37.9 g	35.62 g
Soy protein	33.8 g	-
Casein	-	25.95 g
Fat	10.3 g	2.71 g
CHO	30.7 g	48.03
Sodium	0.13 g	-
Energy, kcal	359	348
Energy, kJ	1501	1455

**EXAMPLE 10**

**Preparation of milk with Abacor without subsequent treatment at very high temperature**

5

Half a liter of low fat (max. 0.3% fat) long-life milk obtained by wholesale was diluted 1:1 with water and 77.5 g of the Verum trial substance (Abacor, Registered trade mark, see table below) was stirred into the liter of thinned milk. The resulting milk preparation contain 25 g of soy protein/L.

10

**Table 5.**

Ingredient	Amount in 100 g Verum trial substance (Abacor REG)
Total protein	37.9 g
Soy protein	33.8 g
Fat	10.3 g
CHO	30.7 g
Sodium	0.13 g
Energy, kcal	359
Energy, kJ	1501



**EXAMPLE 11**

The effect of treatment at very high temperature on protein subunit composition as observed by PAGE.

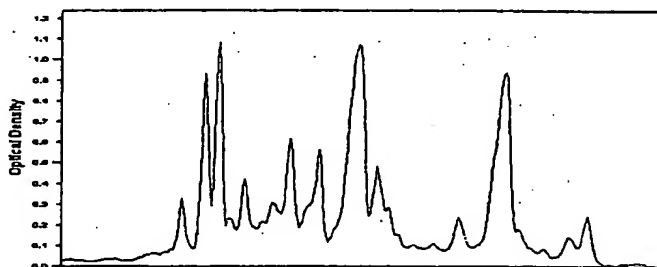
5

An analysis was performed on the soy protein product (Supro 760), used for the preparation in examples 9 and 10. This was compared to an analysis of the preparation from example 9 which had been treated at very high temperature, in order to investigate whether the soy protein subunit composition was changed following heat

10 treatment. The PAGE was performed under reducing conditions.

**Figure 2. Supro 760 analysed by gel electrophoresis.**

The profile shows distinct bands for the 7S and 11S globular proteins.

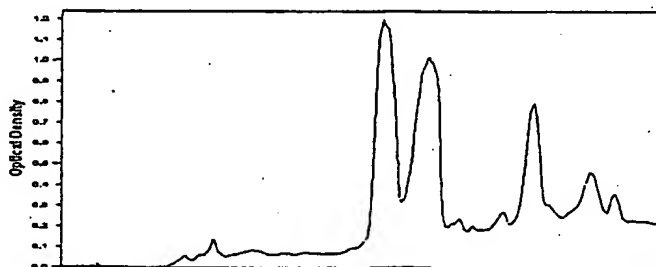


15

**Figure 3. Supro 760 added to milk and treated at very high temperature according to example 9.**

The distinct bands for the globular soy proteins have been considerably reduced. When considering the amount of soy protein applied to each of the lanes, it can be concluded that the bands for the globular soy proteins have been reduced more than half.

20



**EXAMPLE 12****Clinical results from ingesting preparations according to EXAMPLE 9**

5 A study was performed to determine the clinical effects of ingesting the soy protein preparation described in EXAMPLE 9, with 80 trial subjects (51 female, 29 male, ages 30 to 70 years) with hypercholesterolemia (inclusion range: 5.2 – 7.8 mmol total cholesterol/l ~200 – 300 mg/dl) in two dosage groups that were each tested against a placebo group. The trial subjects each drank one liter (dosage group 1), or half a liter  
10 (dosage group 2) of a preparation according to EXAMPLE 9, corresponding to 25 g and 12.5 g of soy protein respectively. For both dosage groups a placebo group was included in which each subject ingested a corresponding amount of the placebo substance according to EXAMPLE 9.

15 Blood samples were taken from the trial subjects at the on-set of the experiment (week 0), at 2 weeks and at 4 weeks (end of the experiment), and total serum cholesterol, LDL cholesterol and HDL cholesterol determined. For the trial parameters the first and third examination comparisons are interpreted by means of the t-test for paired observation and description on the level of a 5% probability level.

20

The data were recorded according to GCP requirements with double data entry and simultaneous value comparison in dBase IV. Study design, performance and documentation of the study was according to the guidelines "good clinical practice" CPMP/ICH/135/95. The primary objective was the reduction of total cholesterol in  
25 serum – secondary objectives were LDL and HDL cholesterol.

Independent of the daily dosage and examination group, in the pre/post comparison it was found that:

- 30
- no effect on the total and HDL cholesterol levels be determined,
  - there was a clear elevation of (16.5 – 18.7 %) the LDL cholesterol levels in serum

35 The resulting lack of effect of the tested soy preparations in chocolate milk on the total and LDL cholesterol was unexpected.

## Results

For both dosage groups 40 subjects each were included. Because there were no premature terminations, the full analysis was performed according to the protocol analysis.

### Total cholesterol (primary objective)

In both dosage groups the verum and placebo groups did not differ in their original values ( $p = 0.48$  relatively  $0.91$ ). During the course of the study there was only marginal change in these values. For dosage group 1 the total serum cholesterol levels were, at the end of the study,  $1.0 \text{ mg/dl}$  ( $0.4 \%$ ) for verum relatively  $4.0 \text{ mg/dl}$  ( $1.6 \%$ ) for placebo lower than at the beginning of the study (difference:  $3.0 \text{ mg/dl}$ ;  $95 \%$  confidence interval:  $-8.2$  to  $+14.2 \text{ mg/dl}$ ), in dosage group 2 the verum values were  $7.9 \text{ mg/dl}$  ( $3.0 \%$ ) lower relatively for placebo with  $1.6 \text{ mg/dl}$  ( $0.6 \%$ ) higher (difference:  $9.5 \text{ mg/dl}$ ;  $95 \%$  confidence interval:  $-33.1$  to  $+14.1 \text{ mg/dl}$ ). This last value was, in the placebo group, strongly influenced by subject Nr. 37 (increase of  $180 \text{ mg/dl}$ ). If this value is removed from the evaluation, the mean value of the placebo group at the 3rd examination was  $253.9 \pm 30.4 \text{ mg/dl}$  and the difference to the 1st examination was ( $261.7 \pm 27 \text{ mg/dl}$  for  $n = 19$ )  $7.8 \text{ mg/dl}$  ( $3.0 \%$ ), thereby there is no difference between the verum and placebo groups.

### LDL cholesterol (secondary objective)

The original situation was homogeneous ( $p = 0.68$  relatively  $0.81$ , s. a. Tab. 9). In both dosage groups the LDL value increased during the course of the study. They were higher in comparison to the original values for dosage group 1 for verum by the end with approximately  $24.9 \text{ mg/dl}$  ( $18.6 \%$ ) and for placebo  $22.7 \text{ mg/dl}$  ( $16.5 \%$ ). In dosage group 2 there was an increase of approximately  $26.4 \text{ mg/dl} = 17.4 \%$  (verum) and approximately  $31.0 \text{ mg/dl} = 20.7 \%$  (placebo). The last named value was (as with the total cholesterol) again by subject Nr. 37 (increase of approximately  $81 \text{ mg/dl}$ ) strongly influenced. Without this value, the mean values at the 3rd examination were  $179.4 \pm 22.6 \text{ mg/dl}$ , and the difference to the 1st examination ( $151.1 \pm 19.8$  for  $n = 19$ ) was  $28.3 \text{ mg/dl}$  ( $18.7 \%$ ).

Therefore, in both dosage groups there was no difference between verum and placebo. It must also be mentioned that for none of the 80 subjects included was there a reduction in LDL serum cholesterol levels, in pre/post comparison.

5 HDL cholesterol (secondary objectives)

With a homogeneous original situation between verum and placebo ( $p = 0.38$  relatively 0.28) the HDL values in the course of the study did not change or only changed minimally (range: - 0.3 to + 2.6 %). The HDL value of subject Nr. 37 was not notable.

10 **Discussion**

The changes in cholesterol parameters found in this study obviously have nothing to do with the effects of soy protein, but in the cases of total and HDL cholesterol lead back to laboratory practice and biological variation. The LDL increased for 78 of 80 subjects (2 subjects had no pre/post change), cannot be explained without further  
15 investigation. Because the subjects were in age, weight, concurrent diseases, concurrent medication, in lifestyle and in the original cholesterol parameters not much different, the unexpected effect could only be due to the production/preparation of the chocolate milk. The low fat milk (fat content approximately 0.3 %) was, together with the additional protein, heated to a high temperature. To clarify this possible cause, a  
20 follow up study is being performed, in which the subjects mix the protein into the milk themselves before use.

Regarding HDL cholesterol, it is consistent with the cited literature that no change is to be expected. Determination of isoflavon content in serum must follow a technique  
25 validation. The serum was therefore stored in a frozen state.

**Conclusion**

The study result showed no effect for soy proteins.

**EXAMPLE 13****Clinical effect of ingesting preparations according to example 10**

- 5 A study was performed to determine the clinical effects of ingesting the soy protein preparation described in EXAMPLE 10, with 20 trial subjects (7 female, 13 male, ages 29 to 70 years) with hypercholesterolemia (inclusion range: 5.2 – 7.8 mmol total cholesterol/l ~200 – 300 mg/dl). The 20 trial subjects each drank one liter of a preparation according to EXAMPLE 10 (25 g of soy protein) daily, for a period of 4 weeks, half a liter with the morning meal and half a liter with the evening meal.
- 10 Blood samples were taken from the trial subjects at the on-set of the experiment (week 0), at 2 weeks and at 4 weeks (end of the experiment), and total serum cholesterol, LDL cholesterol and HDL cholesterol determined. For the trial parameters the first and third examination comparisons are interpreted by means of the t-test for paired observation and description on the level of a 5% probability level.
- 15 The data were recorded according to GCP requirements with double data entry and simultaneous value comparison in dBase IV. The results are shown in the following table:

**Table 6.**

Examination	Total cholesterol	LDL cholesterol	HDL cholesterol
Week 0	252.4±26.3	164.7±22.8	58.0±14.9
Week 2	240.9±23.5	135.2±18.3	55.9±14.2
Week 4	241.6±20.9	148.4±19.0	56.1±14.5
Difference week 2-week 0	-11.5±21.3	-29.5±16.3	-2.1±8.2
p-value	0.031	<0.001	0.27
Difference (%) Week 2-week 0	-4.6	-17.9	-3.6
Difference week 4-week 0	-10.8±18.3	-16.3±16.9	-1.9±5.3
p-value	0.020	0.001	0.13
Difference (%) Week 4-week 0	-4.3	-9.9	-3.3

20

Cholesterol levels are in mg/dl unless otherwise indicated.

**EXAMPLE 14**

**Clinical effect of ingesting undenatured soy protein in drinks according to the present invention.**

5

Two Isolated soy protein products A (SuproSoy), and B (Undenatured ISP) were studied with a placebo C (casein) in a randomized placebo-controlled trial according GCP with respect to their lipid lowering effects.

- 10 Study details: The patients took for 8 weeks daily 25g of the 3 products in 2 dosages in the morning and in the evening. The products were dissolved in water. Intermediate visits occurred after 2 and 4 weeks.

- Patients: 120 patients of both sexes (73 women and 47 men in the age of 32 to 70  
15 were included if the fulfilled the inclusion criteria of 200-300mg/dl or 5.2-7.8 mmol/l total-cholesterol.

- Statistics: For the primary (total cholesterol) and secondary parameters (LDL- and HDLcholesterol) the mean differences between the first and 4th visit weretested using  
20 the group comparison analysis of variance.

- Drop-outs: During the study the following drop outs occurred: Group A: 8 cases, group B: 10 cases and group C: 11 cases, from those only partial results are available. Therefore the analysis was done for the per protocol group, in addition a intention-to-  
25 treat analysis was performed.

Side effects: No severe side-effects occurred.

- Description of patients: Included: 120 patients, finished per protocol: 91  
30

Results: Of the protocol analysis:

The mean age was 55.1 years

weight: the weight increase during the study was 0.2-0.6 kg

35

1) total cholesterol:

Difference visit 4 vs visit 1:

5    A: - 12.8 mg/dl    -5.0%  
     B: - 24.3 mg/dl    -9.4%  
     C: + 1.1 mg/dl    +0.4%

Percentage changes Active vs Placebo:

10

A-C: -5.4%  
B-C: -9.8%

Significances:

15

A:B 0.017  
A:C 0.013  
B:C 0.001

## 20    2) LDL cholesterol

Difference visit 4 vs visit 1:

     A: -12.3 mg/dl    - 7.5%  
25    B: -19.4 mg/dl    -11.8%  
     C: - 5.8 mg/dl    - 3.6%

Percentages changes Active vs Placebo:

30    A-C: 3.9 %  
     B-C: 8.2 %

Significances:

35    A:B 0.081  
     A:C 0.159

B:C 0.006

3) HDL cholesterol

5

No significant changes occurred

Summary:

10

The new ISP formulation of Nutri Pharma has proven to be significantly more effective to reduce total cholesterol than SuproSoy ISP, LDL cholesterol was significantly lowered with Nutri Pharma's ISP but not for SuproSoy, in 91 patients with hypercholesterolemia in 8 weeks in a randomized

15 placebo-controlled trial.

The percentage improvement of total cholesterol of Nutri Pharmas ISP was 9.8% compared to SuproSoy ISP with 5.4%, an improvement of over 80%.

The improvement of LDL cholesterol was 8.2% for Nutri Pharmas ISP compared to 3.9% for SuproSoy, an improvement of over 100%.



## CLAIMS

1. A food product containing soy protein in which the content of intact 7S subunits ( $\alpha + \alpha' + \beta$ ) and 11S subunits (A + B) constitute more than 30 % of the total soy  
5 protein content.
2. A food product containing soy protein in which the content of intact 7S subunits ( $\alpha + \alpha' + \beta$ ) constitute more than 15% of the total soy protein content.
- 10 3. A food product containing soy protein in which the content of intact 7S subunit  $\alpha$  subunit constitute more than 5 % of the total soy protein content.
4. A food product containing soy protein in which the content of intact 7S subunit  $\alpha'$  subunit constitute more than 5 % of the total soy protein content.
- 15 5. A food product containing soy protein, in which the soy protein 7S enthalpy peak is  $>0.5$  J/g soy protein.
6. A food product containing soy protein in which the solubility of the soy proteins in  
20 water is  $>50\%$ .
7. A process for the manufacture of a food product comprising the use of a soy protein product with a solubility in water of  $>84\%$ .
- 25 8. A process for the manufacture of a food product comprising the use of a soy protein product with a 7S enthalpy peak of  $>1,5$  J/g protein.
9. A process for the manufacture of a food product comprising the use of a soy protein product with a 7S enthalpy peak of  $>1$  J/g protein and a protein content of  
30  $>60\%$ .
10. A process for the manufacture of a food product comprising the use of a soy protein product in which the content of intact 7S subunits ( $\alpha + \alpha' + \beta$ ) and 11S (A+B) constitute more than 62% of the total protein content of the soy protein product.

11. A process for the manufacture of a food product comprising the use of a soy protein product in which the content of intact 7S subunits ( $\alpha + \alpha' + \beta$ ) constitute more than 31% of the total protein content of the soy protein product.
- 5 12. A process for the manufacture of a food product comprising the use of a soy protein product in which the content of intact 7S subunit  $\alpha$  constitute more than 10% of the total protein content of the soy protein product.
- 10 13. A process for the manufacture of a food product comprising the use of a soy protein product in which the content of intact 7S subunit  $\alpha'$  constitute more than 8% of the total protein content of the soy protein product.
- 15 14. A process for the manufacture of a food product comprising preparation of the food under conditions rendering the content of intact 7S subunits ( $\alpha + \alpha' + \beta$ ) and 11S (A+B) constitute more than 30% of the total soy protein content of the food product.
- 20 15. A process for the manufacture of a food product comprising preparation of the food under conditions rendering the content of intact 7S subunits ( $\alpha + \alpha' + \beta$ ) constitute more than 15 % of the total soy protein content of the food product.
- 25 16. A process for the manufacture of a food product comprising preparation of the food under conditions rendering the content of intact 7S subunit  $\alpha$  constitute more than 5 % of the total soy protein content of the food product.
- 30 17. A process for the manufacture of a food product comprising preparation of the food under conditions rendering the content of intact 7S subunit  $\alpha'$  constitute more than 5 % of the total soy protein content of the food product.
- 35 18. A process for the conservation of a food product comprising conditions rendering the content of intact 7S subunits ( $\alpha + \alpha' + \beta$ ) and 11S (A+B) constitute more than 30 % of the total soy protein content of the food product.
19. A process for the conservation of a food product comprising conditions rendering the content of intact 7S subunits ( $\alpha + \alpha' + \beta$ ) constitute more than 15 % of the total soy protein content of the food product.

20. A process for the conservation of a food product comprising conditions rendering the content of intact 7S subunit  $\alpha$  constitute more than 5 % of the total soy protein content of the food product.
- 5 21. A process for the conservation of a food product comprising conditions rendering the content of intact 7S subunit  $\alpha'$  constitute more than 5 % of the total soy protein content of the food product.
- 10 22. A process according to any of claims 7 to 21 comprising the use of a soy protein product with a protein content of more than 60% of dry matter.
23. A process according to any of claims 7 to 21 comprising the use of a soy protein product with a protein content of more than 70% of dry matter
- 15 24. A process according to any of claims 7 to 21 comprising the use of a soy protein product with a protein content of more than 80% of dry matter
25. A process according to any of claims 7 to 21 comprising the use of a soy protein product with a protein content of more than 90% of dry matter.
- 20 26. A process according to any of claims 7 to 25 comprising the use of a soy protein product in which the content of active trypsin inhibitor has been reduced to less than 20% of the amount present in the original soy bean material.
- 25 27. A process according to any of claims 7 to 26 comprising the use of a soy protein product in which the arginine:lysine ratio is at least 1.
28. A process according to any of claims 7 to 27 comprising the use of a soy protein product in which the isoflavone content is at least 0.1 % (w/w) of the total soy protein.
- 30 29. A food product containing soy protein produced according to any of claims 7-28.
- 30 30. A food product containing soy protein according to any of claims 1-6 and 29 in which soy protein comprises 0-10 wt% of the dry matter in the food.

31. A food product containing soy according to any of claims 1-6 and 29 comprising more than 10 wt% of the dry matter in the food.
- 5 32. A food product containing a soy protein product according to any of claims 1-6 and 29-31, in which the soy protein is incorporated in the form of soy particles/beads.
33. A food product containing a soy protein product according to claim 32, in which the temperature during the granulation is below 70°C at a humidity of the beads of
- 10 <90%, below 100°C at a humidity of the beads of 60% or below 130°C at a humidity of the beads of < 30%.
34. A food product containing a soy protein product according to claim 32 or 33, in which the beads are dried to a humidity below 20%.
- 15 35. A food product containing a soy protein product according to any of claims 32-34, in which the soy particles have a size between 200 to 10000 microns and a bulk density of 100 to 1300 gram per liter.
- 20 36. A food product containing a soy protein product according to any of claims 1-6 and 29-35 comprising a fermented food product.
37. A food product containing a soy protein product according to any of claims 1-6 and 29-36 comprising a bread.
- 25 38. A food product containing a soy protein product according to any of claims 1-8 and 32-35 comprising bars.
39. A food product containing a soy protein product according to any of claims 1-6 and
- 30 29-36 comprising drinks.
40. Use of a food product according to any of claims 1-6 and 29-39 for preventing, treating, prophylactically treating and/or alleviating a cardiovascular disease.

41. Use of a food product according to any of claims 1-8 and 32-39 in the manufacture of a functional food for preventing, treating, prophylactically treating and/or alleviating a cardiovascular disease.
- 5 42. Use according to claim 40 or 41 where said cardiovascular disease is selected from the group comprising hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, myocardial infarction, and hypertension
- 10 43. Use according to claim 42 where said cardiovascular disease is arteriosclerosis.
44. Use according to claim 42 where said cardiovascular disease is atherosclerosis.
45. Use according to any of claims 40 to 44 where the food product is effective in  
15 lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or increasing the serum HDL/LDL-cholesterol ratio and/or increasing serum levels of HDL-cholesterol and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or preventing, reducing or eliminating fatty  
20 streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation in a subject and/or in reducing or eliminating the risk of a subject contracting angina pectoris and/or reducing or eliminating the risk of a subject contracting a myocardial infarction.
- 25 46. Use of a food product according to any of claims 1-8 and 32-39 for treating a subject suffering from type 2 diabetes and/or the metabolic syndrome.
47. Use of a food product according to any of claims 1-8 and 32-39 in the manufacture of a functional food for treating a subject suffering from type 2 diabetes and/or the  
30 metabolic syndrome.
48. Use of a food product according to any of claims 1-8 and 32-39 for treating a cardiovascular disease in a diabetic subject.
- 35 49. Use of a food product according to any of claims 1-8 and 32-39 in the manufacture of a functional food for treating a cardiovascular disease in a diabetic subject.

50. Use according to any of claims 46 to 49 where the food product is effective in lowering serum levels of glucose and/or of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels and/or glucose tolerance and/or insulin sensitivity and/or alleviating impaired glucose tolerance and/or insulin secretory failure and/or improving insulin secretion.

51. Use of a food product according to any of claims 1-8 and 32-39 for preventing, treating, prophylactically treating and/or alleviating a pulmonary disease in a subject.

52. Use of a food product according to any of claims 1-8 and 32-39 in the manufacture of a functional food for preventing, treating, prophylactically treating and/or alleviating a pulmonary disease in a subject.

53. Use according to claim 51 or 52, where the pulmonary disease is selected from the group comprising inflammation of the airways, bronchoconstriction, bronchitis, asthma, and small airways diseases.

54. Use according to any of claims 51 to 53 where the food product is effective in preventing, treating, prophylactically treating and/or alleviating asthma.

55. Use according to any of claims 51 to 53 where the food product is effective in preventing, treating, prophylactically treating and/or alleviating inflammation of the airways.

56. Use according to any of claims 51 to 53 where the food product is effective in preventing, treating, prophylactically treating and/or alleviating bronchoconstriction.

57. Use according to any of claims 51 to 53 where the food product is effective in reducing and/or eliminating mucus hypersecretion and/or dyspnea in a subject suffering from asthma.

58. Use according to any of claims 51 to 53 where the food product is effective in increasing FEV<sub>1</sub> as measured by forced expiratory volume in the first second of expiration.

59. Use of a food product according to any of claims 1-8 and 32-39 as a nutritional preparation for lowering serum levels of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or for increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels in a subject.

60. Use of a food product according to any of claims 1-8 and 32-39 in the manufacture of a nutritional preparation for lowering serum levels of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or for increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels in a subject.

61. Use of a food product according to any of claims 1-8 and 32-39 as a nutritional preparation for lowering serum levels of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or for increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels in a diabetic subject.

62. Use of a food product according to any of claims 1-8 and 32-39 in the manufacture of a nutritional preparation for lowering serum levels of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or for increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels in a diabetic subject.

63. Use of a food product according to any of claims 1-8 and 32-39 as a nutritional preparation for alleviating a pulmonary condition.

64. Use of a food product according to any of claims 1-8 and 32-39 in the manufacture of a nutritional preparation for alleviating a pulmonary condition.

65. Use according to any of claims 59 to 64 where the nutritional preparation is in the form of a dietary supplement.

66. Use of a food product according to any of claims 1-8 and 32-39 as a partial or total diet for an overweight subject.

67. Use of a food product according to any of claims 1-8 and 32-39 as a partial or total diet for an overweight subject suffering from an arteriosclerotic condition.

68. Use of a food product according to any of claims 1-8 and 32-39 as a partial or total diet for an overweight subject suffering from a diabetic condition.

5 69. Use of a food product according to any of claims 1-8 and 32-39 for preventing, treating, prophylactically treating and/or alleviating a cardiovascular disease in the human or animal body in an amount effective in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels  
10 and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or preventing, reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or reducing or eliminating the risk of a subject  
15 contracting angina pectoris and/or reducing or eliminating the risk of a subject contracting a myocardial infarction, and/or alleviating the clinical condition of patients contracting a myocardial infection.

70. Use according to claim 69 where the cardiovascular disease is selected from the  
20 group comprising hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, myocardial infarction, and hypertension.

71. Use according to claim 70 where the cardiovascular disease is arteriosclerosis.  
25

72. Use according to claim 70 where the cardiovascular disease is atherosclerosis.

73. Use of a food product according to any of claims 1-8 and 32-39 for preventing and/or treating type 2 diabetes in an amount effective in lowering serum levels of  
30 glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or improving glucose tolerance and/or increasing insulin sensitivity and/or alleviating impaired glucose tolerance and/or improving insulin secretion and/or  
35 reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion



formation and/or preventing, reducing or eliminating the risk of a subject contracting angina pectoris and/or preventing, reducing or eliminating the risk of a subject contracting a myocardial infarction and/or preventing, treating, prophylactically treating, alleviating and/or eliminating hypertension and/or hyperglycemia and/or  
5 hyperinsulinemia and/or hypercholesterolemia and/or hypertriglyceridemia and/or arteriosclerosis and/or atherosclerosis and/or arteriolosclerosis in a diabetic subject.

74. Use of a food product according to any of claims 1-8 and 32-39 for preventing and/or treating the metabolic syndrome in an amount effective in lowering serum levels  
10 of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or improving glucose tolerance and/or increasing insulin sensitivity and/or alleviating impaired glucose tolerance and/or improving insulin secretion and/or  
15 reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or preventing, reducing or eliminating the risk of a subject contracting angina pectoris and/or preventing, reducing or eliminating the risk of a subject contracting a myocardial infarction.

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75. Use of a food product according to any of claims 1-8 and 32-39 for preventing, treating, prophylactically treating and/or alleviating a pulmonary disease in a human or animal body in an amount effective in preventing, treating, prophylactically treating and/or alleviating inflammation of the airways and/or bronchoconstriction and/or  
25 bronchitis and/or small airways diseases and/or asthma and/or reducing and/or eliminating mucus hypersecretion and/or dyspnea in a subject suffering from asthma and/or increasing FEV<sub>1</sub> of a subject as measured by forced expiratory volume in the first second of expiration.

30 76. Use according to claim 75 where the pulmonary disease is selected from the group comprising inflammation of the airways, bronchoconstriction, bronchitis, asthma, and small airways diseases.

77. Use according to claim 75 or 76 in an amount effective in preventing, treating,  
35 prophylactically treating and/or alleviating asthma.

78. Use according to claim 75 or 76 in an amount effective in reducing and/or eliminating mucus hypersecretion and/or dyspnea in a subject suffering from asthma.

79. Use according to claim 75 or 76 in an amount effective in increasing FEV<sub>1</sub> of a subject as measured by forced expiratory volume in the first second of expiration.

80. Use according to claim 75 or 76 in an amount effective in reducing inflammation of the airways.

81. A method of preventing, treating, prophylactically treating and/or alleviating by therapy a cardiovascular disease in a human or animal body, said method comprising administration to said human or animal body of a food product according to any of claims 1-8 and 32-39 in an amount effective in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or preventing, reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or reducing or eliminating the risk of a subject contracting angina pectoris and/or reducing or eliminating the risk of a subject contracting a myocardial infarction and/or alleviating the clinical condition of patients contracting a myocardial infection.

82. A method according to claim 81 wherein the cardiovascular disease is an arteriosclerotic condition of the human or animal body.

83. A method according to claim 81 wherein the cardiovascular diseases is selected from the group comprising hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, myocardial infarction, and hypertension.

84. A method according to claim 83 wherein the cardiovascular disease is arteriosclerosis.

85. A method according to claim 83 wherein the cardiovascular disease is atherosclerosis.

86. Method of preventing and/or treating by therapy type 2 diabetes in a human or animal body, said method comprising administration to said human or animal body of a food product according to any of claims 1-8 and 32-39 in an amount effective in lowering serum levels of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or improving glucose tolerance and/or increasing insulin sensitivity and/or alleviating impaired glucose tolerance and/or improving insulin secretion and/or reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or preventing, reducing or eliminating the risk of a diabetic subject contracting angina pectoris and/or preventing, reducing or eliminating the risk of a diabetic subject contracting a myocardial infarction and/or preventing, treating, prophylactically treating, alleviating and/or eliminating hypertension and/or hyperglycemia and/or hyperinsulinemia and/or hypercholesterolemia and/or hypertriglyceridemia and/or arteriosclerosis and/or atherosclerosis and/or arteriolosclerosis in a diabetic subject.

87. Method of preventing and/or treating by therapy the metabolic syndrome in a human or animal body, said method comprising administration to said human or animal body of a food product according to any of claims 1-8 and 32-39 in an amount effective in lowering serum levels of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or improving glucose tolerance and/or increasing insulin sensitivity and/or alleviating impaired glucose tolerance and/or improving insulin secretion and/or reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or preventing, reducing or eliminating the risk of a subject contracting angina pectoris and/or preventing, reducing or eliminating the risk of a subject contracting a myocardial infarction.

88. A method of preventing, treating, prophylactically treating and/or alleviating by therapy a pulmonary disease in a human or animal body, said method comprising administration to said human or animal body of a food product according to any of claims 1-8 and 32-39 in an amount effective in preventing, treating, prophylactically  
5 treating and/or alleviating inflammation of the airways and/or bronchoconstriction and/or bronchitis and/or asthma and/or small airways diseases and/or reducing and/or eliminating mucus hypersecretion and/or dyspnea in a subject suffering from asthma and/or increasing FEV<sub>1</sub> of a subject as measured by forced expiratory volume in the first second of expiration.
- 10 89. A method according to claim 88 wherein the pulmonary disease is selected from the group comprising inflammation of the airways, bronchoconstriction, bronchitis, asthma, and small airways diseases.
- 15 90. A method according to claim 88 or 89 wherein the soy protein product is effective in preventing, treating, prophylactically treating and/or alleviating asthma.
91. A method according to claim 88 or 89 wherein the soy protein product is effective in reducing and/or eliminating mucus hypersecretion and/or dyspnea in a subject  
20 suffering from asthma.
92. A method according to claim 88 or 89 wherein the soy protein product is effective in increasing FEV<sub>1</sub> of a subject as measured by forced expiratory volume in the first second of expiration.
- 25 93. A method according to claim 88 or 89 wherein the soy protein product is effective in reducing inflammation of the airways.

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**FIGURE 1** Flow-sheet, production procedure: